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Non-invasive techniques for the investigation of dermatological diseases

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Background. In recent years, non-invasive physico-chemical techniques were used in medical areas in order to develop new strategies that can help replacing the invasive methods, for instance biopsy. In this context, non-invasive techniques such as Scanning Electron Microscopy (SEM), Energy-Dispersive X-ray Spectroscopy (EDX), X-ray Photoelectron Spectroscopy (XPS), and Attenuated Total Reflection-Fourier Transform Infrared Spectroscopy (ATR-FTIR) have proved their usefulness in the investigation of different diseases, as they can offer qualitative and/or quantitative information regarding changes that occur at the surface of materials[1,2]. In this work two case studies are presented: one is focused on the obtaining high-resolution microscopic images and elemental analysis of a severe onychodystrophy caused by synthetic nails and acrylic adhesives, and the second one is centralized on the determination of the degradation mechanism induced by the psoriasis in human fingernail using SEM, EDX, XPS, and ATR-FTIR techniques.

Materials and methods. SEM and EDX methods were used in the investigation of damaged fingernails by the use of acrylate glue and synthetic nails. SEM, EDX, XPS, and ATR-FTIR techniques were applied in order to obtain information regarding the degradation mechanism induced by the psoriasis in human fingernail from a chemical point of view.

Results and discussions. It was proved through SEM and EDX techniques that synthetic nails, acrylic glue, and nails damaged by contact with acrylate glue have a different morphology and composition compared to healthy human nails. For the case of nail psoriasis, the results obtained by using SEM, EDX, XPS, and ATR-FTIR methods showed that are differences in the chemical structure, elemental composition and surface morphology of healthy and psoriatic fingernails clippings.

Conclusions. The results obtained were complementary and consistently demonstrated that the above mentioned non-invasive techniques could help in the development and optimization of non-invasive diagnostic methods and new treatments.

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Keywords: non-invasive techniques, severe onychodystrophy, nail psoriasis.

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Cryptococcus – an update on epidemiology, taxonomy, and pathogenesis

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Cryptococcus is a basidiomycetous yeast that causes hundreds of thousands of deaths worldwide every year[1]. The main species are *C. neoformans* and *C. gattii,* but the nomenclature of the *Cryptococcus* genus has recently been revised. The disease produced by this yeast, cryptococcosis, affects especially immunocompromised patients but also immunocompetent hosts.

C. neoformans is mostly reported from immunocompromised patients (e.g. HIV positive), has an affinity for the central nervous system, is distributed all over the world, and can be isolated mainly from pigeons and other bird droppings, trees, and soil[2].

C. gattii can also determine infections in immunocompetent hosts, has a predilection for the lung, is prevalent in tropical and subtropical regions, and can be isolated especially form trees (eucalyptus) but also from domestic and wild animals. Since 2000 *C. gattii* has become endemic in Vancouver Island, mainland Canada and the northwestern part of the USA[2,3].

Besides its polysaccharide capsule which constitutes the main virulence factor of *Cryptococcus*, other such factors are some components of its cell wall (chitin and melanin), a broad enzymatic equipment (e.g. laccase, urease, extracellular DNase, superoxide dismutases, phospholipases, proteases), with some differences between species[4,5]. Alongside these factors, *Cryptococcus* has developed immune evasion strategies, both in immunocompetent and in immunocompromised patients[6].

Until 2015, the *Cryptococcus neoformans* complex comprised 2 species: *C. neoformans* and *C. gattii*, divided into *C. neoformans* serotypes A (*C. neoformans* var. *grubii*), D (*C. neoformans* var. *neoformans*) and A/D, and *C. gattii* serotypes B and C. Due to genetic heterogeneity demonstrated by phylogenetic analysis and many different molecular methods, a new classification was proposed for *C. gattii*/*neoformans* species complex, containing 7 species, with different biochemical properties, pathogenicity, and geographical distribution[7,8] .

The aim of this work is to present an update on epidemiology, taxonomy, and pathogenesis of the *Cryptococcus gattii*/*neoformans* species complex.

Keywords: Cryptococcus taxonomy, epidemiology, cryptococcosis pathogenesis

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Influenza-associated aspergillosis

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Background: Both in immunocompromised and in non-immunocompromised patients, viral infections may generate fungal superinfections.

The aim of this presentation is raising awareness regarding the possibility of *Aspergillus spp* infection in severe cases of influenza.

Methods: We reviewed data published on influenza-associated aspergillosis in critically ill patients.

Results and discussions: The circulating respiratory viruses (mainly respiratory syncytial virus and Influenza) were associated with invasive pulmonary aspergillosis) (IPA) and a lower airborne mould spore load was required for IPA to occur during the circulation of the respiratory viruses. Climatic conditions have been found to be associated with a higher risk of IPA in one report, but other studies failed to find an association. A preexisting underlying condition was identified in most patients (mainly corticosteroids treatment), but not the classic underlying conditions predisposing to aspergillosis. In addition, there are cases in patients without any predisposing circumstances. Two recent studies on influenza-associated aspergillosis in intensive care unit setting found this condition in 23/144 (16%) and 21/124 (17%).

The absence of the classic underlying risk factors together with the atypical presentation result in a delayed diagnosis and may conduct to a high mortality exceeding the mortality of severe influenza (varying from 33% in a study published in 2012 to more than 60% in the two studies previously mentioned), despite the antiviral and antifungal treatment. Although these superinfections occur predominantly during influenza A (especially H1N1) infection, influenza B may equal the severity of influenza A infection.

In a multicenter observational case-control study performed by the Dutch-Belgian Mycoses study group the authors found that influenza and corticosteroids were associated with IPA.

Some studies showed no benefit and even potential harm for corticosteroids during severe influenza. The mechanism of IPA during influenza is not clear. Anatomical alterations (disruption of muco-ciliary clearing, uncovering basal membrane, reduction of epithelial cytokine response) together with immunological alterations due to influenza, but permissive for *Aspergillus* growth, and possibly host genetics might play a role.

Conclusion: In severe influenza setting aspergillosis may occur even in immunocompetent patients and rapid diagnosis is needed since the mortality is high even in patients treated with anti-fungal therapy.

Keywords: aspergillosis, influenza, severe, intensive care unit

Determination of fungal colonization index and fungal score - important link from bedside assessment to prevention strategy in patients with risk of fungal infections

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Background: An adequate strategy for early prevention of invasive fungal infections (IFI) has not been established. Candida colonization index on skin/mucosa and Candida score in high risk patients (HRP) residing in intensive care units have contributed significantly to the IFI prediction but similar strategy for invasive mold infections, including invasive aspergillosis is lacking. In this study, we aimed: (i) to create new ready to use culture triple-plates based assay for detection of fungal colonization of mucosa in patients with chronic rhino-sinusitis (CRS) in order to determine the "mold colonization index/ MCI"; (ii) to create scoring platform for "mold score/MS" based on patient data.

Materials and methods: New non-swab sampling method with mucolytic pretreatment on sino-nasal mucosa and lavage was applied in 77 CRS patients from our registry in order to determine "MCI". The patient data were recorded, scored (low, 0; middle, 1; strong, 2), and "MS" was determined based on: SNOT-22 test for QoL, polyp, surgery, radiology/CT, eosinophilia/IgE, and skin test for fungal inhalator allergens.

Results and discussions: Non-swab sampling method with lavage and ready to use culture plates improved detection of fungi on sino-nasal mucosa and proved fungal CRS prevalence in 20.8% (16/77) patients. Using the "MCI", the most common determined strains in Serbian patients with CRS were: Aspergillus (A.) flavus (9/16), A. fumigatus (4/16), Alternaria alternata (2/16), and Cladosporium sp. (1/16). The regression analyses were applied in these patient's, and ten "major fungal criteria" were selected and "MS" was developed. MSindex with ≥ 5 strong fungal criteria tested for prediction of mold infection and complex hybrid algorithm based on "MCI" and "MC" developed. In order to simplify this, the online platform "E.sinonalas Labnet" was developed as a model for early prediction of mold related IFI in HRP.

Conclusions: Fungi in the sinuses are „hidden killers"for HRP, so this assay can be promising in patient selection guiding and decision of starting early anti-mold prophylaxis or therapy. The potential role of "MCI" and "MS" as bedside assessment and "point of impact testing" should be evaluate in immunosuppressed patients with sinusitis.

Keywords: mold colonization index, mold score, high risk patients, fungal infections

Toxigenic molds and mycotoxins in food and agricultural commodities – prevention and control strategies

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Mycotoxins are low-molecular weight natural products produced as secondary metabolites by toxigenic filamentous fungi that contaminate food, the food chain, and represent a risk to human and animal health. The major mycotoxins that occur in food and agricultural commodities are produced by *Fusarium* (deoxynivalenol, trichothecenes, fumonisins and zearalenone), *Alternaria* (alternariol, altenuene, tenuazonic acid), *Aspergillus* and/or *Penicillium* (aflatoxins, ochratoxin A, patulin). Mycotoxins have been shown to be the number one threat amongst food and feed contaminants regarding chronic toxicity. Moreover, the presence of mycotoxins in agricultural products is also an economic concern. A quarter of the world's crops are estimated to be contaminated to some extent with mycotoxins.

Under certain storage conditions, fungi can cause spoilage in stored crop seeds, decreasing crop value, or produce mycotoxins that have a direct effect on human health. Protecting stored wheat grain from fungal spoilage is an essential part of their production. Wheat associated microorganisms can have beneficial effects on the stored grain's health. Understanding the composition and role of stored wheat grain microbiota is crucial toward agricultural practices that are less dependent on chemical fungicides, which has known negative effects on the environment and human health. To explore and characterize microbial communities of stored crop seeds we used amplicon-based next-generation sequencing with the 16S and 18S rRNA genes. A large number of bacterial and yeasts isolates from epiphytic and endophytic microflora of wheat grains was obtained and assessed for their antifungal activity. The results indicate that some of the screened isolates presented antagonistic properties against a variety of mycotoxigenic fungal pathogens. Furthermore, our laboratory is focusing on implementation of molecular biology techniques and analytical methods for rapid detection and identification of mycotoxigenic fungi and mycotoxins in wide range of agricultural commodities. This approach will enable to evaluate the mycotoxicological risk of stored wheat grains, to minimize economic losses and reduce the hazard to animal and human health.

Post-flood indoor occurrence of toxigenic Aspergilli from the *Versicolores* clade: is it dangerous?

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Background. Two years after the flood in Gunja (eastern Croatia) majority of the houses have been repaired but fungal indoor colonisation is still present and may represent health risk (1,2). In winter and summer of 2016 and 2017 samples of airborne and dustborne fungi along with dust were collected in repaired houses of Gunja in order to explore seasonal variations of Aspergilli indoor levels, Aspergilli mycotoxin-producing capacity and mycotoxins in dust, in contrast to control village Gornji Stupnik. Aspergilli (*Versicolores*) were among dominant Aspergilli at both locations and majority of isolates produced sterigmatocystin (STC) and 5-methoxy-STC and (2).

Materials and methods. Here we present; 1) species diversity (calmodulin sequence-based methods); 2) presence of STC and 5-methoxy-STC in the dust (multitoxin HPLC/MS/MS method); 3) cytotoxicity (MTT test), genotoxicity (alkaline comet test) and immunomodulatory effects (ELISA) of STC *vs* STC-producing Aspergilli using human lung A549 cells and THP-1 cells macrophage-like cells.

Results and discussion. *A. jensenii*, *A. creber*, *A. puulaauensis*, *A. griseoaurantiacus* and *A. sydowi* were determined so far (ongoing project). *A. jensenii* was chosen for preliminary experiments on cells. Highest concentration of STC (0.59 mg/g) and 5-metoxy-STC (7.70 mg/g) in dust were detected in winter (2017) in Gunja (Fig.1). THP-1 cells (IC_{50} = 0.6 mg/ml) were twice as sensitive to STC than A549 cells (IC_{50} = 1.3 mg/ml); dose-response for *A. jensenii* extract in both cell lines was similar (IC_{50}>3.2 μg/ml). Subcytotoxic concentrations of STC (0.032 and 0.32 mg/ml) and *A. jensenii* containing the same concentrations of STC showed different immunomodulatory pattern in THP-1 cells; STC induced significant concentration-dependent increase of IL-1b, IL-6 and IL-8 and decrease of TNF-α, while *A. jensenii* did not significantly affect cytokine levels compared to control (0.1% DMSO). Alkaline comet assay showed that STC alone evoked concentration-dependent increase of DNA damage (tail intensity) in A549 cells; both concentrations of *A. jensenii* also significantly increased tail intensity in comparison to control but lower concentration provoked greater DNA damage.

Conclusion. Differences in toxicity pattern of single STC and *A. jensenii* may be explained by the presence of 5-metoxy-STC and possibly other metabolites which might have antagonised STC toxicity.

Keywords: *Aspergillus jensenii*, sterigmatocystin, airborne fungi, cytotoxicity, genotoxicity

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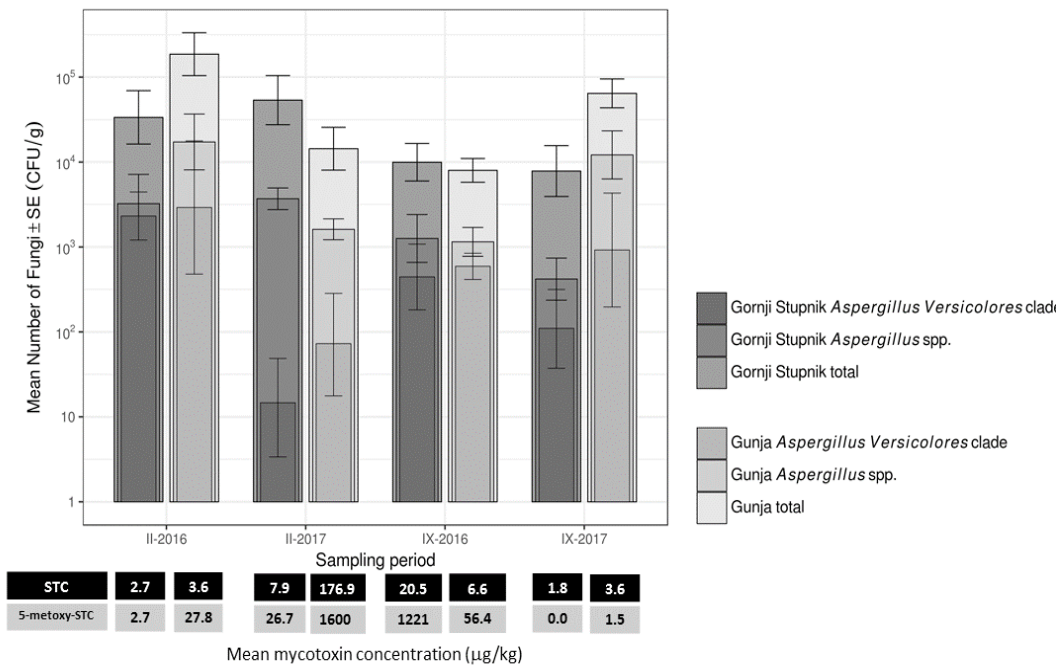

Figure 1. Abundance of Aspergilli from Versicolores clade in total number of dustborne fungi along with detected concentrations of STC and 5-metoxy-STC in Gunja and Gornji Stupnik. In Gunja (SN=24) and Gornji Stupnik (SN=24) dust samples were colected at 5 houses and 1 elementary school during each sampling period.

New trends in rapid diagnosis of superficial fungal infections – could we get over conventional methods?

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Background: Superficial fungal infections (SFI) of the keratin-rich host structures (e.g. hair, nails, and skin) and mucosal (oropharyngeal, vulvovaginal, and intestinal) fungal infections represent the dominant infections worldwide. In recent years, the development of rapid molecular and immunochromatographic (IC) kits for direct detection of the causative agent in the patient's material has led to the emergence of new trends in medical mycology. The aim of this study is to report available data of some rapid assays for the diagnosis of SFI and to highlight the advantages and disadvantages of new diagnostic principles.

Materials and methods: Data for this report were obtained through searches of PubMed using combinations of the following terms: mycological diagnostics, lateral flow assays, immunochromatographic assays, multiplex-PCR, superficial fungal infections, dermatophytes, *Candida* spp..

Results and discussions: So far, there have been reports of IC kits in which antigens of different the most common dermatophyte species of genus *Trichophyton*, *Microsporum* and *Epidermophyton* can be detected in skin and nail material. Besides, IC strip which uses the colloidal gold-anti-mannan IgG conjugate for *Candida* detection in vaginal swabs was also reported. Because of lower diagnostic performances, IC kits are currently recommended only for screening of SFIs, while definitive diagnosis must be confirmed by conventional methods. Contrary, recently published studies showed that real-time PCR with specific pan-dermatophyte primers for detection of agents in samples, which can be completed in a few hours, is highly sensitive and specific. However, the disadvantages of real-time PCR include the high cost of reagents and instruments, and the need for appropriately trained staff.

Conclusions: In next period, we could expect that improvement followed by commercialization of molecular and IC tests will completely change the diagnostics of SFI.

Keywords: superficial fungal infections, dermatophytes, *Candida* spp., molecular assays, immunochromatography

Strategies in designing polymers for transfection

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Gene therapy is an emerging field in modern medicine that promises to treat serious genetic diseases inherited or acquired such as cystic fibrosis, muscular dystrophy, hemophilia, or cancer. Conceptually, gene therapy involves the introduction of nucleic acids into cells, tissues or the body in order to compensate for malfunctioning genes. Gene therapy finds its applicability also in the treatment of fungal infections either by genetically modifying the immune system cells to recognize and to attack pathogens[1] or by training cells at the genetic level to produce more enzymes useful in antifungal defense[2].

Due to their versatility, non-viral vectors based on cationic polymers are the focus of scientists' attention. In this work we have enlisted several concepts in the design of polymeric constructs as carriers for nucleic acids. Our strategy involves the use of core structures such as fullerene, siloxane or β-cyclodextrin which are decorated with polycationic moieties like polyethyleneimine (PEI) and polyethylene glycol (PEG) or it involves Dynamic Constitutional Frameworks (DFC) which are self-rearranging as needed thanks to reversible chemical bonds and interaction with nucleic acids.

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Dynamic constitutional systems used for drugs and genes delivery

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Background: Over the last decades, many treatment strategies against genetic disorders were developed, however many existing therapies lack specificity.[1] The Dynamic Constitutional Systems (DCS's) have shown a promising behaviour in this regard, due to evolutional approach to produce chemical diversity and possibility to self-adjust to biological target species at a given time, in a certain environment at nanoscale dimensions.[2],[3] Another important feature of these systems that once formed, have the ability to reassembled by reversible exchange of components.[4] When a lipid is used in these systems, in the aqueous environment, the system forms the core-shell particles with the hydrophobic core surrounded by a hydrophilic shell.[5] **Materials and methods:** For this purpose, were obtained libraries of aqueous self-assembled DCS's as carriers for nucleic acids delivery which are composed from squalene moiety (natural biocompatible lipid), benzene-1,3,5-tricarboxaldehyde (TA) (multifunctional core), branched polyethylenimine (PEI) (positively charged polymer) and linear polyethylene glycol (PEG) (biocompatible polymer) assembled together by imine bond chemistry. **Results and discussion:** The principle of this study was to obtain the most efficient transfection system by tuning the molar ratios of components which are used to build the systems. TEM and CMC studies showed that in aqueous media this type of systems adopts a core-shell structure by applying dynamic combinatorial chemistry [6]. The formation of the polyplexes between plasmid nucleic acid and DCS's was proved by GelRed assay, showing that, the obtained systems are able to full bind the plasmid nucleic acid at lower N/P ratios of 3. The efficiency in transfection and cytotoxicity were tested *in vitro* on HeLa cell line and results showed that the content of PEG in obtained polyplexes possess a crucial role in delivering genetic material to HeLa cells. **Conclusions:** A new library of DCS's was obtained and characterized. The increasing of PEG molar ratio is leading to higher transfection efficiency as proved by *in vitro* biological assessment.

Keywords: Squalene, drug delivery, gene therapy, self-assembly, micelle, dynamic constitutional systems, polymeric carriers.

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G-Quartet hydrogels for biomedical applications

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Background. Supramolecular hydrogels have found increasing use in tissue engineering and cell growth applications as they display a range of unique physicochemical properties that include water-retention ability, drug loading capacity, biodegradability and biocompatibility[1].

Materials and methods. Herein we report a new G-quartet hydrogel formed from natural guanosine cross linked with benzene-1,4-diboronic acid using K^+ as templating cation (BDBA hydrogel), further cross-linked with Mg^{2+} 2. The G-quartet formation inside the hydrogel structure was confirmed by following the characteristic signals in circular dichroism, also, the gel morphology was evidenced using atomic force microscopy (AFM) and scanning electron microscopy (SEM). Cell viability on the BDBA hydrogels was evaluated using a colorimetric cell proliferation assay. In order to further stabilize and increase the mechanical proprieties of the BDBA hydrogel, a different approach was elected, involving the incorporation of single wall carbon nanotubes (SWCNT) in the gel. The obtained gel was characterized using AFM, SEM, RAMAN and rheological measurements.

Results and discussions. The colorimetric cell proliferation assay showed a good viability of NHDF (normal human dermal fibroblasts) cells at the hydrogel surface. The improved BDBA hydrogel, not only showed great mechanical proprieties, good water retention ability, but also revealed the successful cell adhesion to the hydrogel's surface. The presence and the viability of the attached NHDF cells on the hydrogel's surface was evidenced using a Live/Dead staining assay that demonstrated the increased number of viable cells. Also, an alternate method was used to visualize the adhered cells: the use of a fluorescent dye that allowed cell monitoring from the seeding moment, represented by a β-CD/indolizinyl-pyridinium salt inclusion complex[3].

Conclusions. These new and improved G-quartet hydrogels showed interesting physical and functional properties, including great water retention ability and good cell viability, making these hydrogels suitable candidates for cell growth applications.

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Keywords: G-quartet, supramolecular hydrogel, cell growth applications, tissue engineering.

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Antimicrobial Properties of G-Quadruplex Guanosine - Single Walled Carbon Nanotubes Hydrogels

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Background. Hydrogels are polymeric materials with capability to retain large amounts of water in their structure and are characterized by a soft and rubber-like consistency. Due to their unique characteristics, including high water content, softness, flexibility and biocompatibility, hydrogels have a great potential to be used in biomedical applications (including drug delivery, tissue engineering, and hyperthermia treatment). **Materials and methods.** Here, we report a facile strategy for the obtaining of hybrid dynamic hydrogels with single-walled carbon nanotubes (SWCNTs) homogeneously incorporated into a supramolecular hydrogel system based on guanosine quartet (G-quartet) assembly. This type of hydrogel was prepared by reacting guanosine with corresponding equivalent of 1,4- benzene diboronic acid (BDBA) in the presence of KOH. Guanine moieties of thus prepared dimers in aqueous solutions have the tendency to reversible self-assemble into organized structures in the presence of K^+ ions. In order to enhance the water retention capability and increase the mechanical proprieties of the BDBA hydrogel, we incorporate single wall carbon nanotubes (SWCNT) in the hydrogel. The obtained gel was characterized using SEM, XRD, RAMAN and rheological measurements. The antimicrobial activity was determined by disk diffusion bioassays against three different reference strains: *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. **Results and discussions.** Addition of the SWNTs to the dynamic hydrogels considerably increases the water retention potential of the hydrogel. The viability tests performed on NHDF (normal human dermal fibroblasts) cells for the SWCNT-hydrogels showed an increase of cell viability procentage compared with the simple hydrogel. The tested hydrogels showed no antimicrobial activity against the reference strains: *E.coli* and *C. albicans*, but proved to have antibacterial activity against *S. aurreus*. **Conclusions.**The obtained results suggest that hydrogel–SWCNTs hybrids shows improved properties, good *in vitro* cell viability and antibacterial activity against *S. aurreus* making these materials promising systems for biomedical purposes. **Acknowledgements:** This work was supported by H2020 ERA Chairs Project no. 667387: SupraChem Lab Laboratory of Supramolecular Chemistry for Adaptive Delivery Systems ERA Chair initiative.

Keywords: G-quartet, supramolecular hydrogel, cell viability, antibacterial activity.

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Biomaterials with strong antimicrobial properties based on dynamic iminochitosan derivatives

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Background: Chitosan is a biopolymer with intrinsic therapeutic properties, reason for which it is intensely studied for being used in biomedicine. From the chemical point of view, chitosan is a linear polysaccharide formed by D-glucosamine and N-Acetyl-D-glucosamine units randomly distributed on the polymeric chain. The presence of amine groups on chitosan brings another advantage, making it a real workbench for the development of dynamic architectures, through the formation of reversible imine linkages. On the other side, chitosan presents weak mechanical properties and the incapacity of maintaining its shape, which represent a drawback for its applicability. Studies demonstrated that these problems may be overcame by obtaining chitosan derivatives under the form of films or hydrogels [1,2].

Materials and methods: All reagents were purchased from Aldrich. The structural characterization was done by FTIR and NMR spectroscopy, while the supramolecular architecture was determined by WXRD. The wettability of the films was investigated by the sessile drop method. The morphology was investigated by SEM.

Results and discussion: The study presents the obtaining of dynamic iminochitosan films or hydrogels with antipathogenic properties, by its acid condensation reaction with biologically active monoaldehydes. FTIR spectroscopy demonstrated the formation of the imine linkages in the resulted systems, while WXRD revealed the 3D layered architecture of the formed biopolymers. Contact angle and surface free energy measurements on the iminochitosan films demonstrated a moderate wettability, which suggests a higher biocompatibility in comparison with chitosan, while the microbiological screening demonstrated their self-defense properties against virulent pathogen agents [3]. From the antipathogenic screening, the chitosan derivative containing 2-formyl-phenyl boronic acid stood out by its strong antifungal properties and that is why we further use it, for the obtaining of hydrogels. By FTIR and NMR spectroscopy, and X-ray diffraction it was demonstrated that the hydrogelation mechanism consisted in the supramolecular ordering of the newly formed imine units. The hydrogels showed elastic properties and highly porous morphology. The investigation of the antifungal properties against *Candida albicans* and *Candida glabrata*, on both planktonic yeasts and biofilm, revealed outstanding activity. They also presented good *in vivo* biocompatibility on fibroblasts. The obtained results recommend these materials for the treatment of Candidiasis [4].

Conclusions: The present study demonstrated that iminochitosan derivatives may represent an interesting class of materials for biomedicine, through their valuable properties, such as controlled architecture, morphology, moderate wettability and last but not least dynamicity.

Keywords: Iminochitosan, hydrogels, antimicrobial, antifungal, dynamic biomaterials

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Chitosan imination - a straight pathway to dynamic antimicrobial biomaterials

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Introduction: In the last decades, infections caused by microorganisms became one of the most serious healthcare related problems. Therefore, the development of active antimicrobial materials for the prevention of pathogen colonization is an urgent need. In order to meet the easy manufacturing and sustainability requirements, material formulations usually include antimicrobial biocompatible polymers. In this context, chitosan is one of the most appropriate candidates because of its intrinsic therapeutic properties and environment safeness [1,2].

Materials and methods: All reagents were purchased from Aldrich and used without further purification. The structural characterization was done using a FTIR Bruker Vertex 70 Spectrophotometer, while the supramolecular characterization using WRXD using a X-ray diffractometer LabXXRD-6000. Film samples were analyzed with a field emission Scanning Elec-tron Microscope SEM EDAX – Quanta 200. The antimicrobial activity was considered on three reference strains - Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 6583 and Candida albicans ATCC 10231.

Results and discussion: The present paper reports the obtaining and characterization of twelve chitosan derivatives films. With the aim of obtaining active antimicrobial materials, chitosan, which is known for its antimicrobial properties, was grafted with different biologically active monoaldehydes by reversible imine linkages. In this way, the resulted biopolymers should present a synergistic effect, by combining the two biologically active compounds: chitosan and the monoaldehydes. Moreover, because of the reversibility of the imine linkage in water, the resulted biomaterials should be able to release the grafted monoaldehydes in the aqueous microbiological environment.

FTIR spectroscopy was used in order to characterize the iminochiosan films from the structural point of view and revealed the formation of imine linkages between the reagents and also some significant changes related to chitosan's conformation – from a stiff coil to a straight chain. WXRD evidenced the layered morphology of the biopolymeric films, a consequence of both imination and transamination reactions and hydrophilic-hydrophobic segregation. Contact angle and surface free energy measurements indicated a higher biocompatibility of the new biopolymers in comparison to chitosan, while the microbiological screening demonstrated the self-defense properties of the obtained biopolymeric films against virulent pathogenic agents.

Conclusions: Chitosan imination leads to imino-chitosan biopolymers with lamellar morphologies and, more than this, it allows the obtaining of dynamic materials able to release the antimicrobial aldehydes in the microbiological culture, in a controlled manner.

Keywords: Chitosan, imine, antimicrobial, dynamic biomaterials

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Azoles-loaded magnetic nanoparticles with antifungal effects

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Background. One of the most challenging problems of modern medical care constitutes biofilm formation by different types of fungi, with the consequence of patient generalized infections and subsequent death. **Materials and methods.** The therapeutically approach of this research uses nanotechnology (magnetic nanoparticles) to provide a local increased antifungal effect on different types of biofilms. The nanoparticulate systems were coated with biosynthesized dextran (1% and 2%) and afterwards functionalized with propiconazole. Both the polymer and the therapeutic agent have known antifungal activity. The antifungal activity was tested against *Candida albicans*. **Results and discussions.** The nanoparticles were characterized structurally, morphologically and biologically. The microbiological tests on *Candida albicans* (in planktonic and biofilm phase) showed a maximum antifungal effect of the drug-loaded systems and also a 77% destruction of biofilm by simple dextran –coated magnetic nanoparticles**. Conclusions.** The magnetic nanosystems showed adequate biological properties with double action, azole against the planktonic yeast and dextran on biofilm formation. **Acknowledgments**: This work was supported by Horizon 2020 WIDESPREAD 2-2014: ERA Chairs Project no 667387: SupraChem Lab Laboratory of Supramolecular Chemistry for Adaptive Delivery Systems ERA Chair initiative

Keywords: magnetic nanoparticles, antifungal effect, dextran, propiconazole, biofilm

Dynamic Constitutional Frameworks (DCFs) as inhibitors for biofilm formation

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Background. The aim of this study was to use dynamic constitutional chemistry, based on three components: (i) core, (ii) linker, (iii) cationic moiety that form a supramolecular dynamic systems (frameworks), in order to test its ability to inhibit the biofilms formed by *Pseudomonas aeruginosa* strain *PA01*.

Materials and methods. In this study we used the principle of Dynamic Combinatorial Chemistry and reversible imine bond formation to obtain supramolecular systems with antibacterial effect. The dynamic constitutional frameworks are based on a mixture of three components: a core (1,3,5 – benzenetryaldehyde - **BTA**); a linker (poly(ethylene glycol) bis(3-aminopropyl) terminated, molecular mass of 1500Da- (**PEG1500)**; and different cationic moieties (polyethyleneimine branched of low molecular weigh (800 Da and 2000 Da) and aminoguanidine hydrochloride, components that self assembles in the fittest structure. The fixed amount of test-bacteria were treated with different concentration of frameworks and incubated for 24 hours at 37°C. The crystal violet assay was used to evaluate the effects of these substances on the biomass of biofilms formed by *Pseudomonas aeruginosa*. **Results and discussions**. In literature is established that the bacteria presents sensitivity to molecules that are positively charged in physiological conditions[1, 2]. Also it is known that some polymeric supramolecular structures present antibacterial properties[3], therefore the developed systems, might be interesting due to their composition and it can be expected to display antibacterial properties as described in the literature. The synthetic pathway was established in the literature, thus ^{1}H-NMR technique confirmed the formation of imine bonds between aldehyde group of the core and the amine groups of the linker and of the cationic moiety[4]. In presented study, we can confirm that the cationic moiety has a great importance in affecting the biomass of the biofilm developed by PA01. It was noticed that the mixture between the BTA and PEG1500 has a weak anti-biomass effect and the effect is increased significantly when the cationic moiety was added. Also, in case of framework BTA-PEG1500-AGUA it can be observed a high anti-biomass efficiency compared to AGUA alone. We showed that Dynamic combinatorial chemistry can be a successful tool to obtain new supramolecular structures that can prevent biofilm formation.

Keywords: imine bond, dynamic combinatorial chemistry, biofilm, *Pseudomonas aeruginosa*.

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Experimental pulmonary response to *A. fumigatus* affects intestinal homeostasis

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Background. Having in mind that pulmonary and intestinal mucosal surfaces are part of common mucosal immune system (CMIS) (1), and in the view of recent indications of association of viral (2,3) or bacterial (4) pulmonary infections with changes in the intestine, possible influence of pulmonary fungal infection on intestinal homeostasis was investigated.

Materials and Methods. The rat model of sublethal pulmonary infection with *A. fumigatus* (human isolate) was used (5). Signs of intestinal inflammation were evaluated by tissue histology, by analysis of antioxidative defense enzyme catalase (CAT), pro-inflammatory cytokines interferon γ (/IFNγ) and interleukin-17 (IL-17) and anti-inflammatory cytokine interleukin-10 (IL-10) in intestinal homogenates and by analysis of major gut-draining (mesenteric) lymph nodes (MLN). The diversity of intestinal microbiota was assessed by denaturing gradient gel electrophoresis (DGGE) coupled with sequencing of DGGE fragments. Intestine was checked for the presence of *A. fumigatus by* PCR.

Results and Discussion. Inflammatory cell infiltration, increased activity of intestinal catalase/CAT) during 7 days of pulmonary infection as well as increased levels of intestinal IFNγ and IL-17 (as opposed to unchanged levels of IL-10) during the two-week period depict intestinal inflammation in rats with pulmonary infection with *A. fumigatus*. It could not be ascribed to the fungus as it was not detected in the intestine of infected rats. Increased production of pro-inflammatory cytokines by MLN lymphocytes point to these lymphoid organs as places of generation of cytokine-producing cells. No changes in histology or cytokine responses was seen in spleen of infected animals, showing lack of systemic but rather intestinal mucosal response to pulmonary infection. Drop of intestinal bacterial microbiota diversity (disappearance of several bacterial bands) was noted early in infection with normalization starting from day seven. From day three, appearance of new bacterial bands (unique to infected individuals, not present in controls) was seen, and some of them are pathogens. Alterations in intestinal bacterial community might have affected intestinal immune tolerance contributing thus to inflammation.

Conclusions. Intestinal dysbiosis during pulmonary infection of rats with *A. fumigatus* is in line with current research of lung-gut cross talk. Clinical implications of these data are unscertain at this moment.

Key words. *Aspergillus* lung infection, rats, intestinal inflammation

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Characterization of *Candida* strains exposed to caspofungin

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Background: Research data on antifungal area revealed that the echinocandin resistance is emerging. During or after the treatment *Candida* strains are gaing resistance. Therefore, it is clear that echinocandin resistance depends on this exposure. We have limited knowledge about the virulence determinants of resistant strains. Demonstration of the possible relationship between drug resistance and the virulence is worth of working.

Materials and methods: The aim of this project was the demonstration of virulence abilities of resistant mutant strains of *Candida* exposed to caspofungin. *Candida albicans* ATCC 10231, *Candida parapsilosis* ATCC 22019 and *Candida glabrata* MYA-2950 reference strains were included. Reference strains were exposed to caspofungin in the Sabouraud dextrose agar plates containing caspofungin at concentration 0,03-16 µg/ml. Exposure mutants were evaluated for FKS gene mutations by DNA sequence analysis and for the virulence determinants. *Galleria mellonella* killing scores, adhesion ability, esterase, phospholipase, secreted aspartyl proteinase production, hemolytic activity and biofilm production were investigated.

Results and discussions: Exposure mutants of *C.albicans* ATCC 10231 were selected on the SDA plates containing 0,48 µg/ml caspofungin, *C. glabrata* ATCC MYA-2950 mutants were 0,18 µg/ml and *C.parapsilosis* ATCC 22019 mutants were 1,48 µg/ml. *FKS* gene mutations were detected on the genomes of the mutant strains. A deletion was detected on the 69. position of FKS gene in caspofungin exposed *C.parapsilosis,* on the 203.position of *C. glabrata.* Adhesion ability was found to be raised in mutant strains. Biofilm production was found to be positive in mutant *C. parapsilosis* strain whereas others all biofilm negative. *C. albicans* ATCC 10231, *C. parapsilosis* ATCC 22019 and mutant *C. parapsilosis* were alpha hemolytic. SAP production was found in *C.albicans* ATCC 10231 (1,4 mm/0,6 mm=2,3) and *C.parapsilosis* mutant (1,3 mm/ 0,6 mm= 2,16). Phospholipase and esterase production were all negative in mutant strains. *G.mellonella* killing scores were not different in mutant strains.

Conclusion: In vitro and in vivo virulence determinants of caspofungin exposed mutants were not differentiated from reference strains. It was concluded that, virulence of *Candida* strains and the resistance to caspofungin is not positively correlated.

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Keywords: *Candida*, caspofungin, resistance, virulence

Virulence factors, antifungal susceptibility profile and possible mechanisms of azole resistance among *Candida tropicalis* clinical isolates, Alexandria, Egypt.

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Background. The incidence of infections caused by non *albicans Candida* (NAC) has increased. NAC demonstrate reduced susceptibility to commonly used antifungal drugs. Among NAC, *Candida tropicalis* (*C. tropicalis*) ranks between third and fourth among the most commonly isolated species. The aim of the study was detection of some virulence factors, determination of antifungal susceptibility profiles and exploring possible mechanisms of azole resistance among *C. tropicalis* isolates from ICU patients in Alexandria, Egypt.

Materials and methods. The study included 71 *C. tropicalis* samples isolated from different ICU patients in Alexandria. Identification and antifungal susceptibility profile testing were performed using VITEK 2 compact system. Virulence factors studies included haemolysin, phospholipase, proteinase and biofilm production. Molecular detection of azole resistance included: CDR1 and MDR1 genes expression by real time PCR as well as sequence analysis of Erg11 gene.

Results and discussions. All isolates showed both hemolysin and proteinase activities while only nine isolates (12.68%) showed phospholipase production. Biofilm formation was demonstrated in 98.59% of tested isolates. Fluconazole and voriconazole non-susceptible isolates represented 42.25% and 36.44% of total isolates respectively. As regards, CDR1 and MDR1 genes expression, only CDR1 gene expression in fluconazole non-susceptible isolates was statistically significantly higher than that in fluconazole susceptible isolates (p=0.002). Sequence analysis of Erg11 gene of 26 isolates showed seven mutations; two missense mutations: A395T (Y132F) & G1390A (G464S) and five silent mutations: T225C, G264A, G1362A, C1464T and T1554C.

Conclusions. This study has highlighted increased trends towards elevated MICs levels of fluconazole and voriconazole among *C. tropicalis*, also it demonstrated some virulence factors and molecular mechanisms involved in azole resistance among *C. tropicalis* isolates in Alexandria, Egypt.

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Pathogenic yeast species in Romania and their susceptibility to azoles and echinocandins

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Background. In a multi-centre study including several Romanian tertiary hospitals, over 500 isolates of pathogenic yeasts from systemic and non-systemic infections were identified and tested for antifungal susceptibility to fluconazole (FLC), voriconazole (VOR), caspofungin (CAS), micafungin (MCA), and anidulafungin (ANI).

Materials and methods. The yeast isolates were identified using routine laboratory methods, ID32C strips, MALDI-TOF MS and DNA analysis. Minimal inhibitory concentrations (MICs) of azoles and echinocandins were assessed and interpreted according to EUCAST guidelines. Minimal fungicidal concentrations (MFC) for echinocandins were determined by plating content from the clear MIC wells. The activity was considered fungicidal at MFC/MIC ≤ 4.

Results and discussions. Over 90% of the isolates belonged to the *Candida* genus. *C. albicans* was the most abundant species accounting for over 50% of the isolates. The non-*Candida* and non-*albicans* species showed decreased FLC susceptibility. *C. krusei* accounted for 48% of the FLC resistant isolates. Resistance to VOR was detected mainly in isolates of *C. glabrata* and *C. tropicalis*. The echinocandin MICs were highly correlated and displayed significant MIC essential agreement. ANI had the highest MICs but it also had the highest rate of fungicidal activity together with MCA. *C. albicans*, *C. glabrata* and *C. krusei* had highest rates of echinocandin and multi-drug resistance. The MICs were weakly correlated with the MFCs.

Conclusions. Non-*albicans Candida* isolates accounted for a large percentage, confirming the worldwide reported trends. Only half of the FLC resistance was acquired, coming from non-*krusei Candida*. For echinocandins, MICs and MFCs seem to depend on different factors. Prophylactic treatment and empiric therapy will be problematic because of echinocandin and multi-drug resistance.

Keywords: Candida, species distribution, echinocandin, azole, resistance, fungicidal, yeast Romanian isolates

In vitro activities of nine antifungal agents against clinical *Kluyveromyces marxianus* (*Candida kefyr*) and *Clavisopra lusitaniae (Candida lusitaniae*) isolates

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Background: Although five *Candida* species (*C.albicans*, *C. glabrata*, *C.parapsilosis sensu stricto*, *C.tropicalis*, and *C.krusei*) account for ≥95% of all candidemia or other forms of invasive candidiasis, less common other species (*Kluyveromyces marxianus*, *Clavispora lusitaniae*) may cause problems, especially among cancer and leukemia patients (1,2,3). The aim of the present study was to evaluate the antifungal activity of nine antifungal agents against a collection of clinical isolates of *K. marxianus and C.lusitaniae* to ensure some foresight into management of these infections.

Materials and methods: *Kluyveromyces marxianus* species isolated from bronchoalveolar lavage (BAL) fluid (n=12), urine (n=4), peritoneal fluid (n=3) and blood (n=2) cultures and *Clavisopra lusitaniae* species isolated from urine (n=3), BAL fluid (n=1), peritoneal fluid (n=3) and blood (n=1) cultures of patients who were hospitalized at Medical Hospital of Erciyes University in Kayseri were included in the study. Isolates were identified by conventional methods and the molecular methodology of DNA sequencing analysis. MICs to antifungals were determinated with Sensititre Yeast One (Trek Diagnostics Systems, USA) according to manufactor's instructions**.**

Results: The ranges of minimum inhibitory concentrations (MICs), geometric mean MICs and MIC_{50} and MIC_{90} values (expressed in μg ml^{-1}) of the 21 *K. marxianus* and six *C. lusitaniae* isolates were detailed in the Table 1. For *K. marxianus*; amphotericin B had the highest geometric mean MIC (1 μg ml^{-1}) and voriconazole had the lowest geometric mean MIC (0.010 μg ml^{-1}). For *C. lusitaniae*; flucytosine had the highest geometric mean MIC (8 μg ml^{-1} and voriconazole had the lowest geometric mean MIC (0.011 μg ml^{-1}).

Conclusion: According to our study, it appears that *K. marxianus* have the propensity to develop resistance to amphotericin B, based on the MICs observed. *C. lusitaniae* appears susceptible to amphotericin B, azole and echinocandin antifungal agents, on the other hand shows high MIC values to flucytosine. Given that this less common yeasts may emerge as an important pathogen in the future, it is reasonable tu study the in vitro activity of antifungal agents as potential options for its treatment. Furthermore, more studies are required by testing large panels of geographically diverse clinical isolates.

Key Words: antifungal susceptibility*, Clavisopra lusitania, Kluyveromyces marxianus,* sequencing

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Table 1: The ranges of minimum inhibitory concentrations (MICs), geometric mean MICs and MIC_{50} and MIC_{90} values

		MIC values (µg/ml)							
Antifungal agent		**Incubation time (24 hour)**					**Incubation time (48 hour)**		
	Candida species	**MIC range**	**GM**	**MIC_{50}**	**MIC_{90}**	**MIC range**	**GM**	**MIC_{50}**	**MIC_{90}**
Amphotericin B									
	C. kefyr (n:21)	0.5-2	1	1	1	1-2	1.935	2	2
	C. lusitaniae (n: 6)	0.12-0.5	0.246	0.25	0.5	0.25-1	0.629	0.5	1
Fluconazole									
	C. kefyr (n:21)	0.12-32	0.238	0.25	0.25	0.12-64	0.423	0.25	0.5
	C. lusitaniae (n: 6)	0.12-4	0.442	0.25	0.5	0.12-4	0.702	0.5	1
Voriconazole									
	C. kefyr (n:21)	0.008-0.5	0.010	0.008	0.008	0.008-2	0.014	0.008	0.015
	C. lusitaniae (n: 6)	0.008-0.06	0.011	0.008	0.008	0.008-0.06	0.015	0.015	0.015
Posaconazole									
	C. kefyr (n:21)	0.015-2	0.039	0.03	0.06	0.03-2	0.064	0.06	0.06
	C. lusitaniae (n: 6)	0.008-0.25	0.021	0.015	0.015	0.015-0.25	0.042	0.03	0.06
Itraconazole									
	C. kefyr (n:21)	0.03-1	0.062	0.06	0.06	0.03-4	0.070	0.06	0.06
	C. lusitaniae (n: 6)	0.03-0.25	0.060	0.06	0.06	0.012-0.25	0.135	0.12	0.12
Caspofungin									
	C. kefyr (n:21)	0.03-0.06	0.046	0.06	0.06	0.03-0.12	0.052	0.06	0.06
	C. lusitaniae (n: 6)	0.015-0.12	0.06	0.06	0.12	0.03-0.5	0.246	0.5	0.5
Anidulafungin									
	C. kefyr (n:21)	0.03-0.25	0.116	0.12	0.12	0.03-0.25	0.133	0.12	0.25
	C. lusitaniae (n: 6)	0.12-0.12	0.12	0.12	0.12	0.12-0.5	0.172	0.12	0.25
Micafungin									
	C. kefyr (n:21)	0.06-0.12	0.062	0.06	0.06	0.06-0.25	0.105	0.12	0.12
	C. lusitaniae (n: 6)	0.03-0.25	0.067	0.06	0.06	0.12-0.25	0.153	0.12	0.25
Flucytosine									
	C. kefyr (n:21)	0.06-4	0.180	0.12	2	0.06-4	0.287	0.12	4
	C. lusitaniae (n: 6)	0.5-64	8	16	64	1-64	17.95	64	64

In vitro activity of olive oil and propolis - olive oil against fluconazole-resistant and fluconazole-susceptible *Candida glabrata* isolates

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Background: In recent years, the incidence of infections caused by Candida glabrata has increased considerably, especially among immunocompromised population who has received fluconazole treatment (1). The aim of this study was to evaluate the in vitro activity of olive oil (OL) and propolis - olive oil (OEP) against C. glabrata isolates exhibiting resistance and sensitivity to fluconazole

Materials and methods: Eighty-six strains identified as C. glabrata by conventional methods and DNA sequencing analysis were included in this study. The propolis sample was collected from Kayseri, Turkey. In vitro antifungal activity of OL (Nutral Terapi Co.), OEP, and fluconazole (FLU) was investigated by the microdilution broth methods according to Clinical Laboratory Standards Institute (CLSI) guidelines M27-A3 for yeast. Final drug content in the microdilution plates ranged between 0.125 to 64 µg/ml for FLU, and from 0.1 to 50 % (v/v) for all of OL and OEP. The minimum inhibitory concentrations (MICs) for propolis were defined as the lowest concentration giving optical clarity. For FLU, MIC was defined as the lowest concentration in which 50% decrease in turbidity as visually is observed (2-4).

Results: At 24 hours when all strains were considered together, MIC range values of OEP, OL, and FLU were between 0.1 to 50 % (v/v), 50 % (v/v), and 1 to 64 µg/ml, respectively. At 48 hours when all strains were considered together, MIC range values of OEP, OL, and FLU were between 0,8 to 50 % (v/v), 50% (v/v), and 2 to 64 µg/ml, respectively (Table1.). It was shown that OEP had same antifungal activity against C. glabrata isolates exhibiting both sensitivity and resistance (included dose-dependent susceptible strains) to fluconazole and the MIC range of OEP for both sensitive and resistance was determined as between 0,2 to 50 % (v/v) and 0,2 to 25 % (v/v) at 24 hours and between 0,8 to 50 % (v/v) and 1.56 to 50 % (v/v) at 48 hours, respectively.

Conclusion: This study demonstrated that OEP has antifungal activity against C. glabrata isolates exhibiting both resistance and sensitivity to fluconazole

Keywords: Antifungal activity, Candida glabrata, olive oil, propolis olive oil, fluconazole

Table 1. MICs values obtained for fluconazole, olive oil, and propolis olive oil at the end of 24 and 48 hours incubation of 88 C. glabrata isolates

	Incubation time (24 hour)				Incubation time (48 hour)			
	MIC [a] values				MIC values			
Antifungal agent	Range	GM [b]	MIC_{50} [c]	MIC_{90} [c]	Range	GM	MIC_{50}	MIC_{90}
Olive oil (%(v/v)								
C.glabrata	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**
Propolis olive oil (%(v/v)								
C.glabrata	**0.2-50**	**9.26**	**12.5**	**25**	**0.8-50**	**22.37**	**25**	**50**
Fluconazole (µg/ml)								
C.glabrata	**8-64**	**9.84**	**8**	**64**	**2-64**	**17.75**	**16**	**64**

[a]MIC, minimal inhibitory concentration; [b] The geometric mean range for MIC, [c]MIC_{50} and MIC_{90}, minimal inhibitory concentration at which 50% and 90%, respectively, of the isolates were inhibited.

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Comparative evaluation of antifungal activity of two poly-phenolic compounds: magnolol and honokiol

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Background: The epidemiology of *Candida* infections changed in recent years and, although *Candida albicans* is still the main cause of infections, a substantial proportion of patients is now infected with non-*albicans Candida* (NAC) [1]. Candida species vary in their susceptibility to the most commonly used current antifungal classes. This, along with the development of acquired resistance during treatment, is becoming a major problem in the management of *Candida* [2]. The present study assessed the antifungal activity of two major phenolic constituents extracted from the bark of *Magnolia officinalis*, namely magnolol and honokiol. Several recent reports demonstrated a high antimicrobial activity for these compounds against several microorganisms such as bacteria and molds, but none investigated the activity against NAC yeasts [3].

Materials and methods: The aim was to determine the minimal inhibitory concentrations (MICs) and the minimal fungicidal concentrations (MFCs), and to calculate the specific statistical parameters (MIC_{50}, MIC_{90}). The MICs were assessed and interpreted according to EUCAST guidelines with a final inoculum of 2.5×10^5 CFU/mL. The MFCs were determined by plating content from the clear MIC wells. A number of 356 clinical isolates of yeasts from various clinical specimens were studied for comparative evaluation of antifungal activity of magnolol and honokiol. The clinical yeasts were collected in hospitals from different regions of Romania and were identified using ID32C strips, MALDI-TOF MS and DNA sequencing. Statistical analysis was done with GraphPad Prism version 7.00 (GraphPad Software, La Jolla, California USA, www.graphpad.com).

Results and discussion: Most often, the MIC_{50} values for magnolol and honokiol were 32.0 μg/ml. The MIC_{90} values were usually one dilution higher. The MIC_{90} values for magnolol were 32.0 μg/ml for *C. parapsilosis, C. krusei* and rare species type, 64.0 μg/ml for *C. albicans, C. tropicalis* and other non-albicans, 256 μg/ml for *C. glabrata*. The MIC_{90} values for honokiol were 64.0 μg/ml for *C. albicans*, *C. parapsilosis*, *C. krusei*, *C. tropicalis* and other non-albicans, and 256 μg/ml for *C. glabrata*. Honokiol had the lowest MFC values. For magnolol the MFC_{50} values were between 32.0 μg/ml for all strains of *Candida albicans* and non-albicans Candida species. The MFC_{90} values for magnolol were the same except *C. parapsilosis* and *C. tropicalis* species with 64.0 μg/ml. For honokiol the MFC_{50} values were 32.0 μg/ml while the MFC_{90} values were 32.0 μg/ml for *C. albicans* and rare species, and 64.0 μg/ml for *C. glabrata*, *C. parapsilosis*, *C. krusei*, *C. tropicalis* and other non-albicans species. Both phenolic constituents of *Magnolia officinalis* demonstrated *in vitro* antifungal activity against *C. albicans* and non-albicans Candida species, in terms of their MICs. Against *C. albicans*, *C. glabrata* and *C. parapsilosis* honokiol had the lowest MICs values.

Conclusions: The obtained results suggest that magnolol and honokiol showed a good activity against yeast clinical isolates and could lead to the development of potentially novel antifungals against *Candida* infection. Considering the MIC and MFC values, honokiol displayed a better and more uniform activity in comparation with magnolol.

Keywords: magnolol, honokiol, antifungal activity, *Candida albicans,* non-*albicans Candida*

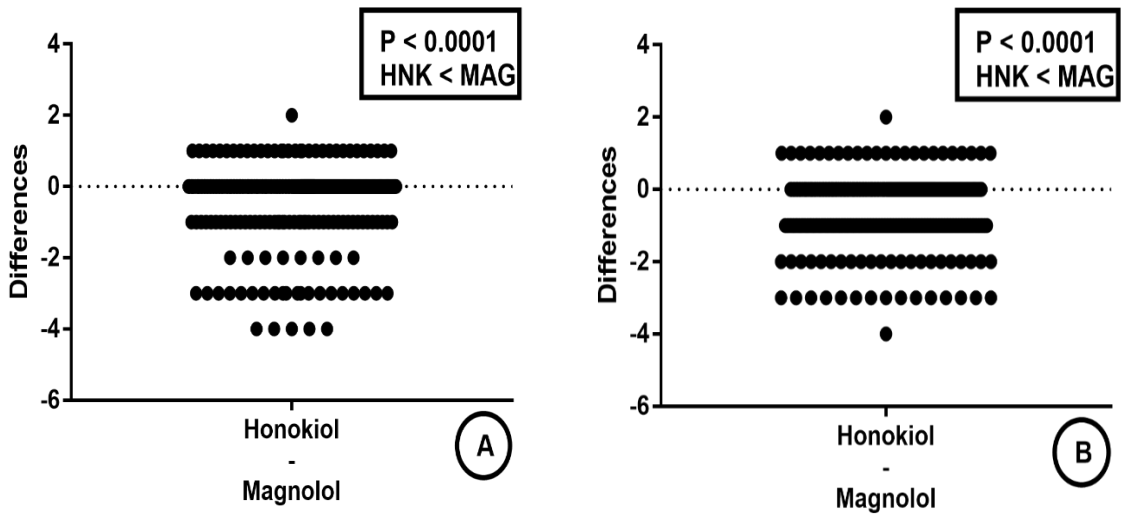

Figure 1. Differences between MAG and HNK MICs (A) and MFCs (B) against all Candida spp. isolates

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Post-traumatic Myositis due to Aspergillus flavus in a Child – Case Report

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Background. *A.flavus* is the second leading cause of invasive aspergillosis and is more virulent than *A. fumigatus*. Myositis and osteitis are associated with A. Flavus following trauma.

Case report. We present the case of an 8-year-old girl who suffered a car accident with subsequent *A. flavus* wound contamination. The girl is admitted to the Iasi Children's Emergency Hospital with traumatic/haemorrhagic shock and with the lower left leg (2/3) on ice. Upon admission, the lower-left limb is replanted with revascularization and a femoral venous graft is initiated. But after 24 hours the infection of the replanted segment occurs.

Muscle-fragment biopsy is taken for microbiological and anatomopathological examination. Gram-smear revealed PMN inflammation and hyphae. Cultivation on Sabouraud agar with chloramphenicol isolated *Aspergillus spp* in 48 hours. At that time the isolate was not identified at species level, nor was susceptibility testing performed. Therefore, treatment with Voriconazole and Amphotericin B was performed instead.

The histological examination of muscle prints shows inflammatory polymorph infiltrate, predominantly PMN, destroyed muscle fibres and septate hyphae with acute angle dichotomous branching.

In spite of general antifungal therapy and rigorous wound dressing 10 days after limb replantation, amputation is required. Further evolution is favourable, subsequent samples for microbiological-anatomopathological examination no longer reveal the presence of *Aspergillus* spp.

We later identified the strain and tested antifungal susceptibility using Yeast One (YO10) Sensititre (TREK Diagnostic Systems). Identification as *Aspergillus flavus* was based on culture (velvety, yellow to green with goldish to red-brown reverse culture, presence of sclerotia) and micromorphological features (Conidiophores variable in dimensions, biseriate/uniseriate, covering the entire vesicle and phialides in all directions; globose or subglobose conidia, conspicuously echinulate). The strain has been shown to have high MICs to fluconazole and amphotericin B (64 and 4 mg/L respectively) and proved to be sensitive to itraconazole, posaconazole, voriconazole (MICs 0,06, 0,03, and 0,25 mg/L respectively) and to echinocandins (caspofungin 0,08 mg/L, anidulafungin and micafungin 0,015 mg/L). Caspofungin could have been a good therapy choice. Although Amphotericin B remains standard treatment in *Aspergillus* invasive infections, amphotericin B resistance is recognized for *A. flavus.* In the case presented, amputation might have been prevented by species identification.

Keywords: *A. flavus*, amputation, Amphotericin B resistance

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Asymptomatic Dermatophyte Scalp Carriage in School Children in Erzincan, Turkey

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Background: This study aimed to investigate the prevalence of symptomatic tinea capitis infections of the scalp and its asymptomatic carriage in students attending primary schools in Erzincan, Turkey.

Materials and Methods: Eighteen primary schools were visited; 1 located in the central district and 17 located in other districts of the Erzincan province. From 2015 November to 2016 April, scalp scrapings were obtained from a total of 1879 students aged 6 to 13 years (mean age: 9.37±1.69) 924 (49.2%) male and 955 (50.8%) female using sterile hairbrushes, and assessed for tinea capitis and asymptomatic fungal carriage. The hairbrushes were used to seed Sabouraud Dextrose Agar containing cycloheximide, chloramphenicol and gentamycin. A questionnaire was students to collect epidemiological data on carriage and infection development.

Results and Discussions: In our study, symptomatic cases were not detected but dermatophyte carriage was detected in a 13-year-old girl of foreign descent (Meskhetian Turk), who had migrated to Erzincan province. The fungal sample was identified as *Trichophyton tonsurans* using the DNA sequencing of the ITS region. When the underlying factors were explored, it was found that the girl was a national wrestler. In the literature, *T. tonsurans* outbreaks have been widely reported in people engaged in combat sports, particularly wrestling and judo. Asymptomatic carriage is that it is mostly caused by anthropophilic dermatophytes (*T. tonsurans*, *T. violaceum*, *Microsporum audouinii*). Our results are consistent with the literature. The prevalence of dermatophyte-positive scalp carriage generally correlates well with the incidence of tinea capitis in community. Symptomatic tinea capitis studies targeting primary school children performed in Adana, Erzurum, Istanbul, Izmir, Diyarbakır, Batman and Afyon reported prevalence of 0.05%, 0.08%, 0.08%, 0.1%, 0.1%, 0.2% and 0.4% respectively. In Erzincan province, the prevalence of asymptomatic dermatophyte carriage observed is 0.05%.

Conclusions: In our study, the prevalence of asymptomatic carrier state was similar with the prevalence of symptomatic cases in Turkey. This study is significant for being the first to investigate tinea capitis infections and carriage in Erzincan, Turkey.

Keywords: Tinea capitis, asymptomatic dermatophyte scalp carriage, *Trichophyton tonsurans*, DNA sequencing analysis.

Activity of different inorganic nanoparticles against fungal isolates colonising buildings included in the Romanian National Heritage

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Background: A current issue in the field of restoration and preservation is the lack of antifungal substances with low or zero health and environmental impact. The purpose of this study is to determine the possibility of using novel compounds to combat fungal strains isolated from buildings included in the Romanian national heritage[1]. The samples were collected during the winter of 2017 from 3 buildings dating from the 19th century, two of them rated class B (local importance) Located in the Neamt County and the third situated in Bucharest, in a protected area, severely affected by a fire, followed by intensive exposure to natural elements. The samples were collected from a wide range of building materials, structural (pillars, beams, walls) and non-structural (cladding, woodwork).

Materials and methods: The isolation and purification of the fungal species was achieved on Sabouraud Dextrose Agar medium. The strains identification was performed by phenotypical examination of culture and morphological features. The molecular identification was done based on the ITS (*Internal Transcribed Spacer*) marker[2], since in the last decade this is a widely used sequence for taxonomy and molecular phylogeny of fungi and other taxa. The following species were identified: *Trichoderma longibrachiatum*, *Penicillum crysogenum, Aspergillus niger, Rhizopus nigricans* . Fourty strains were tested for their susceptibility to different types of inorganic nanoparticles and complex combinations of bivalent metals in binary dilutions[3], ranging from 1 to 0.0009 mg, using a microplate dilution assay. For this purpose, chemical compounds stock solutions of 10 mg/ml performed in DMSO and Sabouraud liquid medium were used.

Results and Conclusions:

The susceptibility assay revealed a mycelium growth and spore maturation inhibition, which was directly proportional with the chemical substance concentration. The collected data is very useful for the development of environmentally safe antifungal substances, which can be used in the control of the fungal biodeterioration process on buildings of cultural importance.

Keywords: cultural heritage, fungi, architecture, biodegradation, nanoparticles

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Human mycoses caused by *Trichoderma*: potential environmental origin

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In accordance with the growing number of immunocompromised patients, the incidence of infections due to opportunistic human fungal pathogens has also been rising substantially (1). Filamentous fungi, such as members of the genus *Trichoderma* are abundant in different environmental habitats (2), but certain species are also known to cause diseases in humans ranging from allergic reactions to localized as well as disseminated infections with even fatal outcome in an increasing number of cases (3). Isolates of these species frequently show resistance to commonly used azole antifungals (4). We present four novel cases of human mycosis caused by *Trichoderma* together with the review of previous studies.

Four *Trichoderma* strains were isolated from human different infections: keratitis in India, otitis externa in Croatia and two cases of endocarditis in Hungary. The isolated fungi were identified by the sequence analysis of fragments of the translation elongation factor 1α (*tef1*) gene, and subsequent phylogenetic studies were performed using *tef1*, internal transcribed spacer (ITS), calmodulin (*cal1*) and hydrophobin 4 (*hfb4*) sequences with the involvement of further clinical and agricultural *Trichoderma* isolates. The antifungal susceptibility of the fungi was determined by the Etest method in comparison with strains recovered from agricultural specimens, while the carbon source utilization profile of clinical and environmental isolates was compared using Biolog Phenotype Microarrays.

Based on their *tef1* sequences all the four novel clinical *Trichoderma* isolates were identified as *T. longibrachiatum*. Neither phylogenetic analysis nor Biolog Phenotype Microarrays revealed characteristic differences between the examined *T. longibrachiatum* strains with clinical and agricultural origin. Most of the studied clinical and agricultural *T. longibrachiatum* isolates could tolerate high concentrations of fluconazole, itraconazole and posaconazole (>256, >32 and >32 μg/ml, respectively).

Our findings confirm that *T. longibrachiatum* is the most prevalent species within the genus *Trichoderma* capable of causing human mycoses, and furthermore suggest that agricultural environments are potential sources of infections caused by this emerging opportunistic fungal pathogen.

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Keywords: otitis externa, keratitis, endocarditis, *Trichoderma longibrachiatum*, azole resistance, agricultural environments

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Mycobiota related to Slovak mummies

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Background. Complex mycological analysis of human remains and their related indoor environments is presented. Moulds belong to the most potent decomposers of organic materials, incl. mummies and skeletons. When being overgrown, these fungi may possess ill health symptoms in occupants dealing with remains (1).

Materials and Methods. Mummies from a nobiliar tomb in Sládkovičovo (20th ct), skeletal remains from crypts under the All Saints church in Sološnica (16th - 18th cts; both Western Slovakia) and under the church of St. Peter de Alcantara (15th - 18th cts; Okoličné, Central Slovakia) were studied. Indoor and related outdoor aeromycobiota was sampled volumetrically and the settled one from the surfaces to perform qualitative and quantitative mycoanalysis. Cultivable mycobiota was identified according to its macro- and micromorphology, and selected microfungi in detail by means of PCR as well. Their proteolytic, lipolytic, esterase and cellulolytic activities were tested due to their potential to act as virulence factors in living organisms (2).

Results and Discussions. From the occupational hygienic point of view, the most striking findings were related to the indoor mycobiotic quantity in the mausoleum in Sládkovičovo with the mummies and in the anthropological laboratory during handling the skeletal remains compraises 960 or 1,095 cfu/m^3 that represented doubled limited count recommended by health care authorities. When judging the qualitative composition of indoor fungal isolates, the presence of toxic aspergilli as well as pathogenic *A. fumigatus* is elevating the possibility of negative health effect occurrence in occupants exposed (3, 4). Any workers manipulating with the mummified and/or skeletal remains have to copy strictly with all preventive measures for handling dangerous biological material and use personal protective facilities, esp. to protect their airways, eyes and skin to minimize ill health symptoms´ occurrence (5).

Conclusions. Deep structural knowledge on tomb indoor fungal colonization enables to employ the effective occupational hygienic preventive measures. As well as, it leads to efficient preservation of the cultural heritage artefacts for the future (6).

Keywords: Human remains, occupational conditions, moulds, aeroscopy, mycotoxins

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Economic impact of mycotoxins on maize chain

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Mycotoxins are the most prevalent contaminants of food and feed worldwide. They are considered an important risk factor for human and animal health. Mycotoxins are secondary metabolites produced by fungi species. Cereals are the most sensitive commodities for growth of toxigenic fungal species and the contamination occurs before harvest (in the field) and after harvest (in storage, during transportation or even processing). The extent of contamination depends on geographic location, agronomic and storage practices, and the vulnerability of the plants to fungal invasion. Food and Agriculture Organization estimates that about 25% of the world's food crops are affected by mycotoxins every year. Maize (*Zea mays* L.) is one of the most important agricultural commodities in the world and is the second most traded cereal (after wheat) in Europe. It is a vital source of food for humans and of feed animals, as well as an ingredient for fuel production or for various industrial applications. The main mycotoxins that affect maize are aflatoxins (B1, B2, G1, G2), ochratoxin A, trichothecenes (especially deoxynivalenol) zearalenone and fumonisins (FB1, FB2) and their maximum limits are regulated in food and recommended in feed by European legislation. Direct economic impact of mycotoxins in maize consists mainly in reducing crop yields and lowering animal performances, meaning additional costs for food and feed supplies, veterinary treatments, reproductive failures, animal weight loss. Indirect economic impact is associated with human health problems due to consuming high quantities of food contaminated with mycotoxins. Direct economic impact is rather easy to evaluate, but demonstrating implication of mycotoxins in illness it is difficult to achieve and more difficult to set certain values.

Keywords: maize, aflatoxins, zearalenone, fumonisins

Genotoxicity and modulation of p53 protein expression in human lung A549 cells upon exposure to single and combined aflatoxin B_1, sterigmatocystin and fumonisins B_1 and B_2

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Background: Aflatoxin B_1 (AFB_1) and its structurally related precursor in biosynthesis sterigmatocystin (STC) are genotoxic liver cancerogens produced by different fungi, namely of the genus *Aspergillus* (1). Liver and kidneys are the target organs of fumonisins, mycotoxins also produced by various fungi. The toxic properties have been well established for FB_1 isoforme that was generally considered a non-genotoxic cancerogen and a promotor of the cancerogenesis with an oxidative stress being a main cause of DNA damage. In more recent studies, it has been found that fumonisin B_2 (FB_2) is an isoforme produced by black Aspergilli (2). The p53 tumor suppressor protein regulates the transcription of numerous genes required for appropriate cellular response to DNA damage. DNA damage induces phosphorylation of p53 at Ser15 promoting both the accumulation and activation of p53 in response to DNA damage (3). Considering the simoultaneus presence of mycotoxins in various indoor environments (4) it is becoming interesting to investigate their effects on the cells originating from the respiratory system in order to estimate their effects upon the inhalation.

Materials and methods: Alkaline comet assay was employed to investigate the genotoxic potencies of AFB_1, STC, FB_1 and FB_2, applied single and in binary mixtures (AFB_1 or STC + FB_1 or FB_2), on human lung adenocarcinoma cells (A549). Upon the same treatment in A549 cells' lysate p53 protein expression (total and phosphorylated at Ser15) was measured by ELISA commercial kit.

Results and discussions: Significant levels of DNA damage were comfirmed for both AFB_1 and STC applied in subcytotoxic concentrations indicating higher genotoxic potential of STC compared to AFB_1. Those actions were supported by elevated levels of both total and phosporilated form of p53 that was not significantly affected upon addition of FB_1 or FB_2, single or in mixtures with AFB_1. However, phospho-Ser15-p53 significantly increased upon simultaneous addition of STC and FB_2.

Conclusions: These results suggest specific and direct genotoxic effect of AFB_1 and STC in A549 cells and antagonistic effect of their binary mixtures with FB_1 or FB_2 moderated by p53.

Keywords: A549 cells, aflatoxin B_1, fumonisins, genotoxicity, p53, phospho-Ser15-p53, sterigmatocystin

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The fungi and mycotoxins in bioterrorism

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Bioterrorism represents the misuse of microorganisms (bacteria, viruses, fungi) or their toxins (mycotoxins, ricin, botulinum toxin etc.) in terrorist purposes (1). In the modern world characterized by global contradictions and great progress in life sciences bioterrorism is recognized as one of the leading security threat with serious potential medical, socio-political, psychological, economic consequences. Fungi as possible bioweapons against humans, livestock, or crops were considered seriously during the Cold War in the biological programmes of the two leading superpowers and have been also attractive for many other countries that developed biological programmes. Fungi cause disease directly by infection or indirectly through production of their mycotoxins. Besides *Coccidioides immitis* that was considered as BSL-3 human pathogen, most of fungi were predominantly considered for use in agroterrorist actions, against plants and animals. The facts that many human pathogenic fungi are easily obtainable in the nature, and can provoke serious disease with relatively low inocula pose them among pathogens that need growing awareness as potential bioweapons (2). Mycotoxins are toxic compounds naturally derived by fungi (*Aspergillus, Fusarium, Penicillium* sp.) that present the biggest chronic health risk when incorporated into the diet (aflatoxins, ochratoxins, fumonisin etc.) As potential bioweapon, T-2 toxin is the greatest concern among them, although a number of other mycotoxins may contaminate a variety of grains and may be lethal in relatively low doses, thus also posing serious threat. The possible intentional mycotoxin contamination of commodities and/or foods could have severe impacts with potential public health outcomes involving high mortality and devastating economic consequences (3). Besides fungi and mycotoxins that can be used as bioweapon due to their characteristics in this paper We also discuss the effective preventive and counter measures against them in the frame of the fight against bioterrorism.

Key words: fungi, mycotoxins, bioterrorism.

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Mycotoxins in foods of non-animal origin – T2 and HT2 toxins

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Background. Trichothecenes are a family of several mycotoxins produced by fungi such as *Fusarium, Trichoderma, Cephalosporium* etc., classified into 4 groups. Group A is the largest and includes T-2 toxin (T2), diacetoxyscirpenol (DAS), neosolaniol and is produced by *Fusarium* species: *F. tricinctum, F. sporotricoides, F. poae, F. equiseti* and is distinguished by the highest acute toxicity. Each fungal species produces more than one toxin in the trichothecenes group, for example *Fusarium tricinctum* produces T2, HT-2 toxin (HT2) and (DAS). T-2 toxin is the most toxic of the group toxins with lethal effects. It is distinguished from the HT-2 toxin by an acetyl group at the C4 position. Both appear simultaneously in infected cereals.

Materials and methods. The results were obtained from 960 analyzed samples over a period between 2013-2018 for T2 and HT2 toxins from raw cereals and cereal-based products of domestic production. The techniques used to obtain the results were immunoenzymatic technique (ELISA) for 912 samples and liquid chromatography with tandem mass spectrometry (LC-MS/MS) for 48 samples.

Results and discussions. From 670 samples of unprocessed cereals, 77% had values <20µg/kg, 5% had values <25µg/kg, 12% had values between 25-50µg/kg and 6% had values >50µg/kg while on processed cereal samples, the results revealed that, of 290 processed cereal samples, 86% had values <20µg/kg, 2% had values <25µg/kg, 8% had values between 25-50µg/kg and 4% had values >50µg/kg.

Conclusions. Unprocessed cereals were 75% contaminated with values >50µg/kg while processed cereal samples were 25% with values >50µg/kg which leads to the conclusion that effective control before grain processing is more effective than in the commercial product phase.

The European Food Safety Authority (EFSA) considers that it is appropiate to evaluate human exposure to the modified forms of the various toxins in addition to the basic compounds. This is due to the fact that many modified forms are hydrolized in the basic compounds or released from the matrix during digestion. For the modified forms of the T2 and HT2 toxins, 10% was added based on reports on the relative contribution of the modified forms.

Keywords: T2 toxin, HT2 toxin, unprocessed cereals, cereal-based products

Molecular identification of some mycotoxigenic fungi

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Molecular methods for the detection of mycotoxigenic fungi from various contaminated feed and food are faster and more reliable than conventional methods. One of the advantage of some methods, especially in PCR-based methods, is the qualitative or quantitative detection of target organisms by amplifying specific DNA sequences from their genome. The most common used methods are derived from conventional PCR, but real-time PCR assays and sequencing DNA methods are now used at large extended. A critical step in molecular methods is the extraction of high quality and quantity of DNA from fungal cultures or from raw materials. In our study several methods for genomic DNA isolation were tested, allowing the obtaining of good quality of DNA. Our interest was focused on *Fusarium graminearum, F.culmorum, Aspergillus flavus, A.ochraceus* and *A.fumigatus* mycotoxigenic fungi as well as the detection of genes coding for mycotoxins production. Specie-specific primers and primers directed for trichothecenes biosynthesis or for aflatoxins production and/or regulation were used in experiments. But applying conventional PCR the identification of *Fusarium* and *Aspergillus* as well as the chemotypes they belonging was possible in short time frame.

Keywords: toxigenic fungi, molecular identification, *Fusarium*, *Aspergillus*, mycotoxins

Antifungal activity of 2-acetylpyridine{n-(4-aminophenyl)-acetamid} thiosemicarbazone and salicylaldehyde{n-(4-amino-phenyl)acetamid} thiosemicarbazone

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Background. Tiosemicarbazones represent a class of widely studied compounds and have been reported in many cases as potential drugs for the treatment of various types of diseases. *Candida albicans* is a fungal species correlated with an important number of symptoms, especially in immunocompromised patients.

The aim of this study is the evaluation of the antifungal properties of 2-acetylpyridine{N-(4-aminophenyl)acetamid}thiosemicarbazone (L^1) and salicylaldehyde{N-(4-aminophenyl)-acetamid}thiosemicarbazone (L^2) - two newly synthesized tiosemicarbazone derivatives, against *Candida albicans*.

Materials and methods. Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) were determined for each substance according to EUCAST standard methods using *Candida albicans* ATCC 10231 as test microorganism. Nystatin was used as control.

Results and discussions. The values of MICs / MFCs for the tested substances were as follows: 31.25 μg/mL / 62.50 μg/mL (L^1), 250 μg/mL / 500 μg/mL (L^2), and 80.00 μg/mL / 80.00 μg/mL (nystatin) respectively.

Conclusions. Both two new thiosemicarbazone derivatives exhibited antifungal activity at various concentrations. The replacement of the pyridinic fragment with the salicylidinic one resulted in a 7.8-8.0 times reduction of MIC and MFC.

Keywords. Thiosemicarbazone derivatives, *Candida albicans*, MIC, MFC

Antifungal properties of new copper (II) complexes with 4-benzoyl-5-methyl-2-phenyl-2,4-dihygro-3*h*-pirazol-3-one n(4)-ciclohexylthiosemicarbazone

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Background. The thiosemicarbazone derivatives are widely studied in medicine for the treatment of various diseases, including fungal diseases. The aim of this work is to synthesize some copper (II) complexes with 4-benzoyl-5-methyl-2-phenyl-2,4-dihygro-3*H*-pirazol-3-one N(4)-ciclohexyl-thiosemicarbazone (L) and to evaluate their antifungal activity against the yeast species *Candida albicans*.

Materials and methods. The thiosemicarbazone derivative (L) was obtained using the condensation reaction between N(4)-cyclohexyl-thiosemicarbazide and 4-benzoyl-5-methyl-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one in ethanol for 2.5 hours at 80°C, after adding 1–2 drops of sulfuric acid. The complexes were obtained by reaction of copper chloride and bromide with the thiosemicarbazone derivative (L) taken in molar ratio of 1:1, in hot ethanol. Sodium hydroxide was added to neutralize the pH and the solution was refluxed for 20 minutes. The complexes ($CuLCl_*C_2H_5OH$ and $CuLBr_*C_2H_5OH$) were obtained as crystalline substances yielding a purity of 95% and 90% respectively. The structure and composition was characterized by elemental and thermal analysis, IR spectroscopy and by X-ray diffraction on mono-crystals. Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) were determined for each substance according to EUCAST standard methods using *Candida albicans* ATCC 10231 as test microorganism.

Results and discussions. The values of MICs / MFCs for the tested substances are presented below: 125 μg/mL / ≥ 1000 μg/mL ($CuLCl_*C_2H_5OH$) and 125 μg/mL / 500 μg/mL ($CuLBr_*C_2H_5OH$) respectively.

Conclusions. New copper (II) complexes with 4-benzoyl-5-methyl-2-phenyl-2,4-dihygro-3*H*-pirazol-3-one N(4)-ciclohexylthiosemicarbazone exhibited antifungal activity but further studies are needed in order to characterize their ability to inhibit fungal growth.

Keywords: Thiosemicarbazone, Copper (II) coordination complexes, antifungal activity, *Candida albicans*

Antifungal activity of some 3d metal coordination compounds with 2-[2-(prop-2-en-1-ylcarbamothioyl)-hydrazinylidene]-propanoic acid

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Background. Despite all advances in new drugs synthesis, the substances exhibiting high biological activity remain an actual problem of modern chemistry. It is mainly caused by widespread dissemination of resistant microorganisms, both bacteria and fungi. In recent years, coordination compounds of biometals with thiosemicarbazones have been actively studied for solving this problem. Many of these substances are biologically active and allow us to use them as a basis for new antimicrobial, anticancer, and anti-tuberculosis drugs, as well as selectively acting microbiological nutrient media, disinfectants or antiseptics. Therefore, the synthesis and study of the biological properties of the new biometal coordination compounds with thiosemicarbazones are of both scientific and practical interest. The aim of this work was the determination of the antifungal activity of coordination compounds of iron, cobalt, nickel and copper with 2-[2-(prop-2-en-1-ylcarbamothioyl)-hydrazinylidene]propanoic acid (H_2L).

The salts of stated above metals form coordination compounds with thiosemicarbazone H_2L with the following composition: $M(HL)_2X$ (M = Fe, Co; X = Cl^-, Br^-, NO_3^-), $Ni(HL)_2$, $Cu(HL)X$ (X = Cl^-, Br^-, NO_3^-) and $CuL(H_2O)$. Their composition and structure were proved using elemental analysis, physico-chemical methods and X-ray diffraction analysis.

Materials and methods. The antifungal activity of the synthesized substances was investigated *in vitro* on yeast species *Candida albicans* (type strain ATCC 10231) using the micro broth dilution technique according to standardized methods.

Results and discussions. The initial thiosemicarbazone (H_2L) exhibited only fungistatic activity at a concentration of 0.5 mg/mL, while all synthesized coordination compounds possessed selective antifungal activity at a range of concentrations between 0.0625 – 0.5 mg/mL. The nature of the central atom and acidic residue has the main influence on the minimal inhibitory and minimal fungicidal concentrations. The antifungal activity decreases in the following way: Cu>Fe»Co>Ni; Br^- > Cl^-» NO_3^-.

Conclusions. These compounds are of interest for medical practice as potential antifungal agents and further studies are needed in order to accurately detect their spectrum of activity and systemic toxicity.

Keywords: antifungal activity, biometal coordination compounds, thiosemicarbazones.

Antifungal activity of iron, cobalt, nickel and zinc coordination compounds with 2-[1-(2,4-dihydroxyphenyl)ethylidene]-*n*-(prop-2-en-1-yl)-hydrazinecarbothioamide

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Background. Thiosemicarbazide derivatives are widely used in medicine in the treatment of various types of diseases. All of them have a wide range of donor atoms and form with metal ions coordination compounds with different composition, structure and properties. In many cases, their biological activity is in good agreement with their structure. Therefore, the synthesis and study of the biological activity of new biometal coordination compounds with similar Schiff bases is of both scientific and practical interest.

The aim of this work was the determination the antifungal activity of coordination compounds of iron, cobalt, nickel and zinc with 2-[1-(2,4-dihydroxyphenyl)ethylidene]-*N*-(prop-2-en-1-yl)hydrazine-carbothioamide (H_2L).

The salts of stated above metals form coordination compounds with thiosemicarbazone H_2L with the following composition: $M(HL)_2X$ (M = Fe, Co; X = Cl^-, NO_3^-), $Ni(HL)X{\cdot}nH_2O$ (X = Cl^-, NO_3^-; n = 2, 3) and $ZnL(H_2O)$. Their composition and structure were proved using elemental analysis and physico-chemical methods.

Materials and methods. The antifungal activity of the synthesized substances was investigated *in vitro* on yeast species *Candida albicans* (type strain ATCC 10231) using the micro broth dilution technique according to standardized methods.

Results and discussions. The initial thiosemicarbazone (H_2L) exhibited only fungistatic activity at a concentration of 0.5 mg/mL, while all synthesized coordination compounds possessed antifungal activity at concentrations ranging between 0.031 – 0.25 mg/mL. The nature of the central atom has the main influence on the minimal inhibitory and minimal fungicidal concentrations. The antifungal activity decreases in the following way: Fe > Ni > Co » Zn.

Conclusions. These compounds are of interest for medical practice as potential antifungal agents and further studies are needed in order to accurately detect their spectrum of activity and systemic toxicity.

Keywords: antifungal activity, biometal coordination compounds, thiosemicarbazones.

Beneficial Effects of Administration of Avian Immunoglobulin (IgY) in a Diabetic Patient with *Staphylococcus aureus* and *Candida albicans* Infected Wound

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Background. In July 2016, patient S.M., aged 68, diagnosed with insulin-dependent diabetes since 1994, suffered a surgical intervention consisting in the amputation of the 3rd gangrenous left lower limb finger. One month after surgery, the surgical wound was still open, developing a purulent fistula. Microbiological analyzes revealed the presence of *Staphylococcus aureus* and *Candida albicans*.

Materials and methods. The therapist from the IMUNOINSTANT Alternative Immunotherapy Practice recommended a protocol consisting of oral and topical (at the fistula level) administration of products obtained from PC2 hyperimmune eggs (1). These contain polyvalent IgY specific for several pathogens, including *Staphylococcus aureus* and *Candida albicans* (2).

The following protocol was recommended:

Orally: purified IgY, aqueous solution, 80 mL/day at a concentration of 200 mg IgY/ 100 mL administered in the evening, before bedtime, for 3 months, and freeze dried whole hyperimmune egg, containing 200 mg IgY/dose, 1 dose/day administered in the morning after breakfast, for 4 months.

Locally: Purified IgY, sterile aqueous solution at a concentration of 200 mg IgY/ 100 mL, sprayed 3 times/day for 3 months.

Results and discussions. Three months after the initiation of the therapeutic protocol, the wound healed completely. Laboratory analyzes performed at the end of the IgY treatment period showed negative results for both pathogens, confirming the effectiveness of IgY in controlling such infections (3,4).

Conclusions. IgY therapy has no adverse effects. The only contraindication is for patients with allergic background, i.e. patients with known allergy to eggs. This is an effective alternative to classical antimicrobial treatment and most likely the only therapeutic option for patients with strains resistant to already established molecules, as well as for patients with various complications such as diabetes, multiple infections, overlapping fungi, other chronic diseases.

Keywords. Avian Immunoglobulin (IgY), *Staphylococcus aureus*, *Candida albicans*

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Avian Immunoglobulin (IgY) Therapy in a Patient with *Proteus mirabilis* and *Candida albicans* Infected Surgical Wound

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Background. In March 2016, B.D. patient, aged 48, was involved in a serious road accident. The injuries suffered, respectively the cranial trauma and the multiple costal fractures, required hospital admission and emergency thoracic surgery. Two weeks after, the surgical wound was still open, with no healing tendency; instead, it developed a purulent fistula. Microbiological analyzes of the collected secretion revealed infection with *Proteus mirabilis* and *Candida albicans*.

Materials and methods. The lack of patient's response to classical antimicrobial therapy and worsening of his general health condition (requiring induced coma) led his family to decide for additional administration of IgY-based products (1). Two months after initiation of the induced coma, the patient was given by gastric tube a sterile aqueous solution of polyvalent IgY, active against the two incriminated pathogens, 40 mL/dose at a concentration of 200 mg IgY/ 100 mL, once every 6 hours, for 5 months (2, 3).

After recovering from 7 months induced coma, the patient continued to ingest immunoglobulin for another 2 months using the following protocol:

purified IgY, aqueous sterile solution, 100 mL/day, at a concentration of 200 mg IgY/ 100 mL, in the evening; whole lyophilized hyperimmune egg, containing 200 mg IgY/dose, 1 dose/day in the morning.

Results and discussions. After 9 months of avian immunoglobulin therapy, the wound healed completely (4). The microbiological tests performed when the patient was discharged showed negative results.

Conclusions. IgY therapy is an effective alternative to classical antimicrobial therapy, especially in the case of resistant isolates and in patients with various complications, such as multiple infections, overlapping fungal infections, multiple organ damage.

Keywords. Avian Immunoglobulin (IgY), *Proteus mirabilis*, *Candida albicans*

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Modification of antioxidant enzyme activity in *Trichophyton mentagrophytes* under the action of spirulina extracts

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Background. Adverse effects, often severe, of the antifungal treatment in combination with high rate of resistance of pathogens dictate the necessity of new formulations intended for treatment of invasive infections caused by fungi. *Arthrospira platensis* (spirulina) is used extensively as a source of protein, but also of substances with high biological activity, including antifungal activity. *Spirulina* biomass enriched with metals could serve as a perspective source in order to obtain efficient formulations for treatment of invasive mycoses. The aim of this study was to highlight the effect of extracts obtained from *spirulina* biomass enriched with metals (Cd, Co and Cr) on antioxidant enzymes of *Trichophyton mentagrophytes* which cause ringworms, and zoonotic skin disease in human.

Materials and methods. The type strain *Trichophyton mentagrophytes* ATCC®9533™ was used. The spirulina extract was obtained from Institute of Microbiology and Biotechnology (Republic of Moldova). The activity of antioxidant enzymes was determined using specialized kits, according to the manufacturer's protocol - SOD kit RANSOD and GPx kit RANSEL (both from RANDOX®LABORATORIES), CT - Catalase Assay Kit (Sigma-Aldrich). The action of the extracts was compared with that of itraconazole and naphthylamine hydrochloride.

Results and discussions. In *Trichophyton* untreated biomass, the activity of SOD was 141.36±18.7 U/mL, CT - 7.34±0.34 U/L, GPx - 1536±47 U/L. Both positive controls and extracts from spirulina (containing metals incorporated into protein structures) produced a reduction in the activity of antioxidant enzymes. SOD activity decreased by 57-62%, CT activity by 68-85%, and GPx activity by 39-45%. One of the mechanisms that ensure the antifungal action of extracts consists in reducing the activity of primary antioxidant enzymes, which are pathogenic factors for fungal cells. The essential decrease in the activity of catalase, superoxide dismutase and glutathione peroxidase leaves fungal cells without protective factors.

Conclusion. The extracts from *spirulina* biomass enriched with metals (Cd, Co and Cr) reduce the activity of antioxidant enzymes in *Trichophyton mentagrophytes.*

Keywords: Spirulina, antifungal activity, antioxidant enzymes, *Trichophyton mentagrophytes*

Construction of an uracil auxotrophic mutant of the opportunistic pathogen *Lictheimia corymbifera* using an *in vitro* CRISPR/Cas9 method

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Background. *Lichtheimia corymbifera* is an opportunistic human pathogenic fungus[1], which can cause primary cutaneous and deep tissue infections in immunocompromised patients. Until now, transformation systems have not been available for the genetic modification of this fungus. Gene deletion or insertion in the genomes of Mucoromycotina species are generally difficult to achieve and the mitotic stability of the transformant colonies is often low[2-4]. The CRISPR/Cas9 system offers a reliable and fast method for genome editing in different organisms and this RNA guided mutagenesis has recently been developed and optimized for another Mucoromycotina species, *Mucor circinelloides*[5].

Materials and methods. In this study, we used a plasmid free CRISPR/Cas9 system to construct a uracil auxotrophic mutant from *L. corymbifera*. PEG-mediated protoplast transformation method was used to introduce the Cas9 enzyme and the synthesized *pyrG* specific guide-RNA (gRNA) into the fungal cells. After the transformation, the protoplasts were inoculated onto YNB minimal media supplemented with uracil and 1.5 mg/ml fluoroorotic acid. The transformation efficiency was 8 colonies per 10^5 protoplast and the genome editing efficiency was 37.5%.

Results and discussion. Molecular analysis of the transformant colonies indicated a three nucleotides gap upstream from the PAM sequence as a consequence of the non-homologous end joining repair of the DNA double strand break. To test the mitotic stability, each transformant was passed several times onto selective and non-selective media and all of them proved to be mitotically stable. We have started to analyze the growing ability of the *pyrG* mutant isolates under different cultivation conditions (e.g., different media, different temperature, oxidative stress).

Conclusions. Our result suggested that the *pyrG* mutant strains has reduced growing ability under different cultivation conditions compared to the wild type.

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Keywords: *Lichtheimia*, CRISPR, Cas9, *pyrG*, PEG

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Candida esophagitis – a risk factor for invasive fungal infections in immunosuppressed patients

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Background: Invasive fungal infections are the price of advancing in healthcare and their incidence increased in the last 20 years (1). The source can be endogenous - from patient mycobiome, with a mechanism called "persorption" (2) using dectin1 inflammasome pathway (3) or exogenous - from environment, health workers' hands, air or food supplements.

Materials and methods: Our retrospective study analyzed the esophageal *Candida* colonization of 1172 patients hospitalized between 2010-2015 at the National Institute of Infectious Diseases in Bucharest (Romania) and the occurrence of invasive fungal infections (detected on blood cultures, tip of the catheter and cerebrospinal fluid) in order to find a relation between colonization and invasion.

Results and discussions: We have diagnosed 55 cases of esophagitis due to *Candida albicans* or other yeasts: 43.63% in patients with HIV infection, 20% with chronic hepatitis, 9.09% liver cirrhosis, 1.81% GERD, 1.81% meningitis, 1.81% pyelonephritis, 1.81%, rheumatic polyarthritis and 18.8% dyspeptic syndrome. We have retrospectively compared 55 patients identified with esophagitis to 79 patients with proven invasive fungal infections in the same period of time. The incidence of invasive fungal infections was 3.7 in 1000 hospitalized patients.

Conclusions: *Candida* esophagitis expresses an evolutional immunosuppression (4), which needs to be explored. Prophylactic therapy with antifungals in patients with risk factors for invasive fungal infections can prevent such diseases.

Keywords: candida esophagitis, invasive fungal infections, *Candida*, HIV, chronic hepatitis

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Exophiala phaeomuriformis endophthalmitis: Case report

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Background. The genus *Exophiala* consists of over 40 different black yeast species. These fungi are causing various uncommon forms of cutaneous, subcutaneous and disseminated human infections, but eye infections due to *Exophiala* species are extremely rare. Most cases occur after a penetrating injury or post-eye surgery.

Materials and Methods. We report a case of 20-year-old patient with postoperative fungal infection in right eye. He was admitted first because of a corneal perforation by a screwdriver hit. The cornea was repaired and topical antibiotics were prescribed. Three months later, the patient was operated for post-traumatic cataract. Microbiological evaluation of aqueous humor and iris tissue samples gave no significant result. In time, despite of intensive medical treatment and serial surgical interventions, the patient developed endophtalmitis. Thus, the patient was undertaken in a new operation including vitrectomy. Culture of vitreous sample yielded a pigmented yeast growth.

Exophiala species was pre-diagnosed. Identification on species level was performed by DNA sequencing. Total genomic DNA was extracted from the yeast colony. The fungal primers ITS1F 5'-CTT GGT CAT TTA GAG GAA GTA-3' and ITS4R 5'-TCC TCC GCT TAT TGA TAT GC-3' were used for amplifying a 500 bp region of the 5.8S rRNA gene. The PCR products were sequenced using an ABI Prism TM 310 Genetic Analyzer (Applied Biosystems, USA) and a BigDye® Terminator Cycle Sequencing Ready Reaction kit (Perkin-Elmer) according to the manufacturer's instructions. The sequence data has been analyzed using the National Center for Biotechnology Information (NCBI, Bethesda, Md., USA) BLAST system (available at http://www.ncbi.nlm.nih.gov/BLAST/).

Results. According the results of conventional and molecular tests, the yeast was identified as *Exophiala phaemuriformis*. The infection was controlled by intensive topical antifungal treatment, however development of phthisis bulbi and total loss of vision could not be prevented.

Conclusion. Endophthalmitis due to *Exophiala phaemuriformis* is a rare but serious infection of the eye. *Exophiala* spp. should be taken into consideration as a causative agent of eye infections.

Key words: *Exophiala phaemuriformis,* mycotic endophthalmitis

Zygomycosis in Firat University Hospital between 2009-2017: A review of five cases

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Background. Despite the ubiquitous availability of the mucormycetes, they cause lethal infections only predisposing conditions occur - diabetes mellitus, hematological malignancies, long-term immunosuppression or corticosteroid therapy (1). The most common clinical form is rhinocerebral involvement. Lung, skin, gastrointestinal tract, central nervous system involvement and rarely disseminated form are also seen (2).

We present 5 cases of mucormycosis which were referred to our hospital between 2009-2017.

Case 1. A diabetic patient who received insulin therapy applied to our hospital with complaints of 2 days, visual impairment, eye pain, abdominal pain, nausea and vomiting. Hemorrhagic, ecchymotic, necrotic black lesions in the base on the right lateral side of the nose have been observed.

Case 2. A 52-year-old man with a history of sinusitis diagnosed with diabetes mellitus and chronic renal failure was admitted to our hospital with fever, headache, visual impairment, nausea and vomiting for 5 days. The patient was debrided with endoscopic sphenoid sinus surgery.

Case 3. A 55-year-old woman suffering of diabetes mellitus and chronic renal failure was admitted to our hospital with fever, facial edema, visual disturbance and eye pain for 5 days. Their lesions were debrided with functional endoscopic sinus surgery.

Case 4. A 72-year-old male patient with diabetes mellitus and chronic renal failure was admitted to our hospital with fever, headache, visual impairment and pain in the right eye area for 3 days. Paranasal sinus tomography showed right maxillary sinus mucosa thickening and bone destruction. The lesion was debrided with functional endoscopic sinus surgery.

Case 5. A 64-year-old male patient with diabetes mellitus and chronic renal failure diagnosed with diabetic ketoacidosis was admitted to our hospital with fever, facial edema, visual disturbance and eye pain for 7 days. The lesions were debrided with endoscopic sinus surgery.

Tissue samples were sent to the relevant departments for histological and microbiological investigation. Microbial and histological examination of the samples revealed unseptated hyphae. The diagnosis of mucormycosis was confirmed.

Conclusions: Mucormycosis is an extremely fast-progressive, fatal, opportunistic fungal infection that is more common in diabetic ketoacidosis and in patients with long-term neutropenia (3).

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Septıc arthrıtıs due to *Trichosporon asahii*

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Background. *Trichosporon* species are etiological agents of either superficial infections such as white piedra or deep trichosporonosis. *Trichosporon asahii (T. asahii)* is the most common cause of deep tissue trichosporonosis and disseminated infections, especially in immunosuppressed patients (1).

In this article, we report a case of septic arthritis produced by *Trichosporon asahii* in joint fluid.

Case report. An 81-year-old male patient with diabetes mellitus and heart disease for approximately 15 years applied to our hospital with a complaint of left-sided pain and swelling. The patient underwent surgery and a 4-cm incision through the lateral side of the left knee has been performed under sedoanalgesia. The acquired material was sent to the microbiology and pathology laboratories with an initial diagnosis of septic arthritis. A fungal culture occurred after 36 hours on Sabouraud Dextrose Agar (SDA). Gram stain smears and lactophenol cotton blue mounts were prepared and examined microscopically. The fungal isolate was identified as *T. asahii*. The pathology report referred to a severe acute inflammatory reaction and fibrinous exudate. Amphotericin B therapy was started, but switched soon to posaconazole because of hypokalemia as side effect. Under antifungal therapy, the septic arthritis of the knee has been cured but the known heart failure exacerbated because of the hemodynamic disorders and acute renal failure.

Dıscussıon. Trichosporonosis is a deep infection caused by yeasts belonging to *Trichosporon* genus. *T. asahii* is the most important species leading to deeply resident infections in humans, with *T. mucoides* being the second one (2,3). Hematological malignancies, wide burns, organ transplantation, use of central venous catheters, use of corticosteroids and peritoneal dialysis are risk factors for invasive *Trichosporon* infections. Although there is no neutropenia, it should not be forgotten that severe infections with *Trichosporon* species may occur when appropriate conditions are encountered in patients with risk factors such as central catheterization, diabetes mellitus or antibiotics use (4). Early diagnosis and empirical treatment are important factors in reducing the mortality because trichosporonosis is usually fatal in the absence of an appropriate treatment (5).

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Pulmonary aspergilloma: report of two cases

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Background: Pulmonary aspergilloma (mycetoma or fungus ball) is the grafting of a superinfection with *Aspergillus fumigatus* (1,2) on an overt tuberculous cavity frequently located in the upper lobes (3). The incidence of this complication is estimated to be between 11-17% (4). Diagnosis confirmation is difficult, from less than a year to 30 years, with an average of 9.2 years (5).

Materials and methods: We present two cases of aspergilloma - one diagnosed two years after tuberculosis and treated both medically and surgically, and the other one discovered post-mortem during the necropsy after a massive hemoptysis. **Results and discussions:** The first case was a 43-year-old man diagnosed in 2000 with upper right lob fibrous–cavitary pulmonary tuberculosis (BAAR positive 2+, with full-class treatment I, cured) and who after 2 years accused the recurrence of respiratory symptoms and haemoptysis, raising suspicion of relapse. After many investigations an aspergilloma was suspected and the patient was treated with antifungal and subsequently surgery. The immediate clinical evolution was complicated (wound superinfection, restrictive pulmonary dysfunction), but very good afterwards. Case 2 was a 63-year-old man with tuberculosis (12 years ago) who was hospitalized for a massive hemoptysis. The treatment was inefficient and the patient died 2 days after admission. Surprisingly, the cause of death at necropsy was aspergilloma associated with multiple bacillary sequelae. **Conclusions:** Aspergilloma is a rare condition, difficult to diagnose and highly unpredictable. Treatment is rather individualized than standardized, however full recovery can only be achieved surgically.

Keywords: aspergilloma, complication, surgical treatment

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Systemic candidiasis with meningeal and digestive determination in a 5 month old pacient

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Background: *Candida parapsilosis* is a human pathogen that has dramatically increased in significance and prevalence over the last two decades, with digestive origin, but also with catheter-related nosocomial transmission and it has become the second most commonly species of candida isolated in blood cultures, causing invasive candidiasis.

Materials and methods: 5 month old patient presented fever, chills, diarrhea, psychomotor agitation, vomiting with onset 2 days ago, and one day ago she had seizures. . She presented at Infectious Diseases Clinic where she was hospitalized for specialized treatment. Clinical examination at admission: general influenced condition, somnolent patient, pale skin, palpebral edema, persistent skin fold, tachycardic heart beats (AV=152 bpm), normal breath sounds, large abdomen, painful at superficial palpation. Positive meningeal signs. She was biological and paraclinical investigated and lumbar puncture was performed. An Intensive Care consultation was also requested and patient was hospitalized in ICU of Infection Disease Clinic the following day.

Results and discussions: Leukocytes 19800/μL, neutrophils 13,6%, lymphocyte 20%, monocytes 11,4%, ALT 108 U/L, AST 209 U/L, creatinine 58 μmol/L, LDH 969 U/L, CRP 34,90 mg/dL. Lumbar puncture: CSF elements 19/mm^3, CSF glucose 4,1 mmol/L, CSF proteins 0,5g/L, negativemicroscopic and bacteriological examination. Cranial CT Scan: centimetric hypodensities located cortico-subcortical bilateral fronto-parieto-occipital. CSF analyses, cerebral imagery suggested meningoencephalitis. Blood-culture: present *Candida parapsilosis*. Stool culture: *Candida parapsilosis* >30 UFC. In ICU she received treatment with: Virolex 100 mg/8h, Meropenem 240 mg/8h, Vancomycin 90 mg/6h, Fluconazole 40mg/day, Arginine sorbitol 20%, 30ml/day, Valproic acid 0.7ml/8h, hydro-electrolytic rebalancing solutions, lactulose-free milk. After 11 days, she was discharged from ICU and continued to be treated in Infectious Diseases Clinic. Evolution was slowly favorable.

Conclusions: This invasive candidiasis had a very probable digestiv tract origin and associated a meningocerebral impairment with minimal CSF abnormalities and cerebral hipodensities – sepsis-related or even with fungal invasion in central nervous system (but we did not identified the fungus in CSF). *Candida parapsilosis* is susceptible to Fluconazole and our isolate met expectations. The very good Fluconazole penetration in CSF provided a favorable course of the case

Keywords: *Candida parapsilosis,* meningoencephalitis, Fluconazole, blood-culture

Tinea capitis in a 9-year-old boy after having a haircut

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Backround: Tinea capitis is the most common superficial fungal infection (dermatophytosis) in children. The disease is caused by a variety of species, for example, zoophilic Microsporum canis predominates in Central and Southern Europe (80%), while anthropophilic Trichophyton tonsurans predominates in the United Kingdom (50-90%), Canada and USA (1, 2). The aim of this paper is to report a case of tinea capitis due to *Trichophyton tonsurans* in a Greek boy after visiting a hair salon.

Case report: A 9-year-old boy presented with a 10 days history of itching and scalp scaling. His general condition was good. Physical examination revealed 2 patches of hair loss accompanied by slightly inflammatory lesions in his scalp in the place where a haircut design (line) was made by an electric haircut machine. Hair plucked from the lesion and scales collected from this site revealed spores and fungi textures by direct microscopy (KOH). Culture on Sabouraud Dextrose Agar revealed *Trochophyton tonsurans* that was identified by microscopical and macroscopical observation of the grown colonies. The patient was treated with oral itraconazole twice a day (daily dose of 4mg/kg), and topical flutrimazole shampoo day per day (3, 4). After one month of treatment the patient recovered and direct microscopy and culture became negative. The use of antifungal shampoo recommended for another two months. Transaminase levels (SGOT, SGPT) were appropriate during treatment period.

Conclusions: Guidelines for disinfection and sterilization of instruments in beauty salons, such as hair salon, is a great need. Although *Microsporum canis* is the dominant cause of tinea capitis in Greek children, doctors should also be alerted for antropophilic dermatophytes because of human migration from endemic areas (5). Fungal culture is essential to confirm and support the diagnosis.

Keywords: tinea capitis, hair salon, *Trichophyton tonsurans*

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Fungal keratitis - report of three cases from Gaziantep-Turkey

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Background: Fungal keratitis is an eye infection with bad prognosis that is difficult to treat and may cause vision loss (1, 2). *Aspergillus* and *Fusarium* species are mold fungi most commonly causing infection in humans (1, 2, 3). These types of filamentous fungi are commonly found in nature and cause severe opportunistic infections in patients with certain risk factors (2). Three keratitis cases were sent from Gaziantep University, Faculty of Medicine, Ophthalmology Clinic to the mycology laboratory from 2015 to 2018 for fungal investigation. The samples of corneal scrape or contact lens were investigated by direct microscopy with potassium hydroxide (KOH). *Aspergillus* spp. in two cases and *Fusarium* spp. in one cases have been isolated in culture.

1st Case (M.Ö): A 34-year old male patient attended our hospital with loss of vision, pain and eye-watering in November 2015 with a history of contact lens use and sleeping with the lenses in. Corneal scrape sample from the patient revealed septated hyphae and *Aspergillus* spp. was isolated in cultures. Treatment of strengthened amphotericin B, voriconazole and caspofungin was administered. In spite of treatment, progression was observed and corneal perforation developed. Penetrating keratoplasty and treatment with strengthened voriconazole+ambisome was used. The patient is in stable condition 2 years post-surgery.

2nd Case (S.A): In January 2016 a 40-year-old male patient attended with pain and vision loss complaints after a branch hit his eye. Corneal scrape sample was investigated in 10-15% KOH and septate hyphae were identified. *Fusarium* spp. was isolated in culture. Treatment of strengthened voriconozole + ambisome was used. With progression in the eye continuing, penetrating keratoplasty was performed. Infection continued after keratoplasty so a second keratoplasty was performed. The patient was administered strengthened voriconozole + amphotericin B and infection control was ensured.

3rd Case (A.T.): A 31-year-old male patient attended our hospital in May 2018 with keratoplasty performed for herpetic keratitis. On the 40th day post-surgery, the patient attended with pain, burning, stinging and watering after milk fresh from milking splashed his eye. Lens sample of the patient revealed septate hyphae and *Aspergillus fumigatus* was isolated in culture. Treatment of re-penetrating keratoplasty + strengthened voriconozole and ambisome was administered. The patient is stable with medical treatment 3 weeks post-surgery.

Conclusion: Though fungal keratitis is rarely observed, it may progress with bad prognosis. As a result, microbiological identification and definition of the vector is very important for accurate treatment.

Keywords: Keratitis, *Aspergillus* spp., *Fusarium* spp.

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Simultaneous mucormycosis and invasive aspergillosis in a patient with relapse acute lymphoblastic leukemia

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Background: Intense chemotherapy regimens, stem cells transplantation (SCT) and related immunosuppression increase the frequency of invasive fungal infections in patients with hematologic malignancy. These can be a significant cause of morbidity and mortality (1, 2). Here, we present a case simultaneous pulmonary mucormycosis and aspergillosis.

Case report: A 15-year old male patient attended Gaziantep University Pediatric Hematology Clinic with complaints of bone pain, fever and night sweats in June 2017. Test results indicated diagnosis of "Pre-B-cell Acute Lymphoblastic Leukemia (ALL)". ALL IC-BFM 2009 chemotherapy protocol was begun. With good response to steroids and no bad prognostic risk factors, the patient was assessed as moderate risk group (MRG). During chemotherapy, no severe infection attack was experienced. In the 9th week of maintenance treatment, pancytopenia was identified (white cell: 290/mm^3, hemoglobin: 12.3 g/dl, platelet: 22,000/mm^3). With peripheral distribution 8%, bone marrow aspiration was 76% blast, and flow cytometry of the patient was in accordance with Pre-B ALL. He was assessed as very early isolated medullary relapse ALL. ALL REZ BFM 2002 chemotherapy protocol was begun. After F1 and F2 block treatments, the patient did not enter remission and had IDA-FLAG and FLAG treatments due to having a fully compliant sister and SCT was planned. In the 1st week of IDA-FLAG treatment the patient had 4 days fever with cough and liposomal amphotericin B was begun as antifungal. Thoracic tomography found frosted glass appearance in the central lobe of the lung and left lung parenchyma. On follow-up serum galactomannan index was 1.98, with worsening general status and falling oxygen saturation, the patient continued with combined antifungal treatment (liposomal amphotericin B + voriconazole). On consecutive tests serum galactomannan index was positive. Broncho-alveolar lavage culture produced *Mucor* sp. on the 3rd day and *Aspergillus fumigatus* on the 5th day. Due to increasing respiratory failure on follow-up, the patient required mechanical ventilator support and died on the 10th day without entering remission.

Conclusion: As the immunosuppression duration lengthens, opportunistic fungal infections are frequently observed in patients. Mucormycosis and invasive aspergillosis are still associated with high mortality in spite of the use of intense and early antifungal treatments (3). Though rare, it should be remembered that two different fungal species may be observed together in the same patient.

Key Words: ALL, Mucormycosis, Aspergillosis

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Fungal infective endocarditis - particularities of evolution and management

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Background. Fungal Infective Endocarditis (FIE) is a rare disease that develops in patients with predisposing cardiac conditions and comorbidities, exhibiting a tremendous progression, severe complications, and reserved prognosis. In spite of modern antimicrobial therapy and heart valve surgery existence the mortality in infective endocarditis (IE) is still remaining 10-20% of cases, and in fungal IE the death rates reach 41-72%.

The objectives of the study were to evaluate the clinical and laboratory features of patients with FIE.

Materials and methods. The prospective study included 289 consecutive patients with IE, hospitalized in specialized Cardiology Departments from four medical centers in Chisinau, Republic of Moldova, during the period 2007 - 2017. The overall characteristics and risk factors in FIE were analyzed.

Results and discussions. Among 289 patients (70.2% men and 28.8% women) with definite IE, 6 (2.1%) patients developed fungal IE, in 4 cases the causative agent was *Candida albicans* and in 2 - *Aspergillus niger* group. The mean age was 51±6 years, with a slight male preponderance (66.7%). The majority 3 patients (50%) had native valve IE, 2 patients (33.3%) – early prosthetic valve IE and in one case (16.7%) was healthcare-associated IE, unfortunately in 66.7% of patients it was diagnosed post-operatively. The predominant risk factors were: rheumatic heart disease in 50%, previous valvular surgery in 33.3%, antibiotic use - 83.3%, and in one case (16.7%) - intravenous drug user. The prevalent comorbidities in these patients were: hepatitis (50%) and diabetes mellitus (33.3%). The aorta was the most common site of the vegetations in 50% cases, and in one case it was detected a valvular abscess. Major complications were: congestive heart failure (83.3%), thromboembolic syndrome (66.7%), with predominant limb arteries affecting, neurological complications and renal failure (33.3%). All patients were treated with antifungals and in 3 patients (50%) the surgical intervention was possible. The overall mortality rate was 50%.

Conclusions. Fungical IE develops more frequently in patients with predisposing cardiac factors and comorbidities, predominantly affecting the aortic valve, with severe complications (thromboembolic syndrome, cardiac and renal failure) and high mortality. Early diagnosed, correct drug therapy and emergency surgery facilitates a favorable prognosis.

Key words: Infective Endocarditis, fungal, complications, antifungal therapy.

Mixed fungemia within 18-years in a university hospital and antifungal susceptibility profile of the isolates

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Background. Fungemia due to more than 2 different species of yeasts (mixed fungemia, MF) is an uncommon and rarely investigated condition (1, 2, 3, 4). This study was conducted to identify the incidence of MF, define the clinical characteristics of patients, and to determine the antifungal susceptibility profile of the isolates.

Materials and Methods. Adult patients with MF between January 2000 and January- 2018 were included. The isolation and identification were done by standard mycological methods (5). Antifungal susceptibility testing (AFST) was performed and evaluated according to CLSI guidelines (6, 7). Individual patient files and medical records were searched for demographic and clinical data.

Results and Discussion. There were 25 patients with 26 MF episodes (Table 1). The incidence of MF among all fungemia episodes was 3.0%. Median age of patients at the time of MF was 55 (range 30-85), and 52% of them were female. Solid organ tumor was the most common (44%) underlying disorder. The mean time to onset of fungemia was 27 days of hospitalization. Presence of central venous catheter, antibacterial therapy, intensive care unit stay and total parenteral nutrition prior to onset of MF was 84%, 80%, 60%, and 60%, respectively. Patients with neutropenia was 24%, and antifungal exposure was 20%. Mortality was 48%. The mean time between the onset of fungemia and death was 30 days. The most common preferred antifungal agent for the initial treatment was fluconazole (46%), followed by an echinocandin (42%). Fluconazole susceptible-dose-dependent (SDD) or resistant *Candida* species were detected in nine episodes (eight *Candida glabrata*, one *Candida krusei*). Available AFST data revealed one fluconazole SDD *Candida parapsilosis* isolate. There was not any resistant strain to echinocandins among *Candida* isolates. However, one episode was caused by non-*Candida* yeasts (*Trichosporon asahi* and *Saprochaete capitata*) which possess intrinsic resistance/reduced susceptibility to both echinocandins and fluconazole.

Conclusions. MF is rare at our institution. The foremost combinations responsible for MF were *C.albicans* - *C.parapsilosis* and *C.albicans* - *C.glabrata*. Echinocandins and fluconazole were mainly preferred for initial treatment. Detection of a mixed infection might offer an opportunity for optimum treatment, in case a fluconazole non-susceptible species or isolate is one of the contributors.

Keywords: Mixed fungemia, *Candida albicans*, *Candida parapsilosis*, *Candida glabrata*, non-*Candida* fungemia, antifungal treatment

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Table: *Characteristics of mixed fungemia episodes*

Episode No.	Patient No.	Sex	Age	Year	Specimens*	Microorganisms	Initial treatment
1	1	M	70	2000	1 B	*C.albicans* + *C.parapsilosis*	Fluconazole
2	2	M	61	2004	1 B	*C.albicans* + *C.glabrata*	Fluconazole
3	3	F	66	2004	1 B	*C.lusitaniae* + *C.kefyr*	Amphotericin B
4	4	M	85	2004	1 B	*C.albicans* + *C.kefyr*	Fluconazole
5	5	F	54	2007	1 B	*C.albicans* + *C.tropicalis*	Fluconazole
6	6	F	46	2007	1 B + 1 C	*C.albicans* + *C.glabrata*	Fluconazole
7	7	F	53	2008	1 B	*C.parapsilosis* + *C.lusitaniae*	Fluconazole
8	8	F	55	2009	1 B	*C.albicans* + *C.parapsilosis*	Fluconazole
9	9	M	58	2010	1 B	*C.parapsilosis* + *C.tropicalis*	Fluconazole
10	10	F	59	2013	6 B + 6 C	*C.albicans* + *C.dubliniensis*	Caspofungin
11	11	M	49	2013	1 B + 1 C	*C.parapsilosis* + *C.lusitaniae*	Fluconazole
12	12	M	47	2013	1 B + 1 C	*C.albicans* + *C.glabrata*	Caspofungin
13	13	F	33	2013	1 B	*C.parapsilosis* + *C.glabrata*	Fluconazole
14	14	M	44	2014	2 B	****C.albicans* + *C.parapsilosis* + *C.guilliermondii*	Caspofungin
15	15	F	61	2014	1 B + 1 C	*C.albicans* + *C.dubliniensis*	Caspofungin
16	16	F	49	2014	2 B + 2 C	*C.albicans* + *C.glabrata*	Fluconazole
17	17	F	70	2014	1 B + 1 C	*C.albicans* + *C.glabrata*	Anidulafungin
18	18	M	40	2014	1 B	*C.albicans* + *C.parapsilosis*	Fluconazole
19	18	M	40	2014	1 B + 1 C	*C.parapsilosis* + *C.glabrata*	Caspofungin
20	19	M	40	2014	1 B + 1 C	*C.krusei* + *C.dubliniensis*	Caspofungin
21	20	M	30	2014	1 B	*C.albicans* + *C.parapsilosis*	Caspofungin
22	21	M	58	2014	1 B	*Saprochaete capitata* + *Trichosporon asahii*	Amphotericin B
23	22	F	63	2015	1 B	*C.albicans* + *C.parapsilosis*	Amphotericin B
24	23	M	72	2015	1 B + 1 C	*C.albicans* + *C.parapsilosis*	Caspofungin
25	24	F	39	2016	1 B	*C.albicans* + *C.kefyr*	Caspofungin
26	25	F	78	2017	1 B	*C.albicans* + *C.glabrata*	Caspofungin

*: B=blood culture obtained from venipuncture, C= blood culture obtained from venous catheter

**: Second blood culture yielded only *C.albicans* + *C.parapsilosis*

Rapid evolutive invasive Aspergillosis in an HIV immunosuppressed patient with Hodgkin's lymphoma

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Background. Invasive aspergillosis (IA) is a life-threating opportunistic infection with low but constant incidence in HIV-infected patients, despite the advent of highly active antiretroviral therapy

Materials and methods. We describe a challenging case due to a rapidly evolving invasive bronchopneumonia with *Aspergillus fumigatus* in an HIV-infected patient and post-mortem diagnosis of Hodgkin's lymphoma.

Results and discussions.The case involves a 46 year-old man diagnosed with HIV stage C3 one year before, who was non-adherent to antiretroviral treatment and showed immunological failure (CD4+ count: 127 cells/mm^3). He had undergone a bone marrow biopsy one month prior for prolonged fever and a potential lymphoma, when he was restarted on tenofovir/emtricitabine/raltegravir treatment. The biopsy had come back negative yet he returned to our clinic for persistent high fever with recent abdominal pain, jaundice and diarrhea. The initial clinical exam revealed cervical lymphadenopathies and grade I splenomegaly, with mild hepatomegaly and no pulmonary rales or cardiac murmurs. Chest X-ray was normal as was the cardiac sonography. Laboratory data indicated a high inflammatory syndrome, severe cholestasis, and negative blood cultures, stool cultures and PCR assays for *Clostridium difficile*. Blood multiplex PCR for bacteria and fungi remained negative.

He subsequently developed severe immunosuppression (CD4+ count: 4 cells/mm^3) and pancytopenia, favoring the onset of various infections (EBV and HHV2/HHV4 reactivation and *Enterococcus faecium* bacteremia). Four days before death he developed respiratory failure unresponsive to aggressive antiviral, antibiotic and antifungal (Fluconazole) treatment. Post-mortem histopathologic samples confirmed Hodgkin's lymphoma and revealed *Aspergillus fumigatus* bronchopneumonia previously undetected by repeated blood cultures and multiplex PCR from blood.

Conclusions. The rapid progression of Hodgkin's lumphoma in HIV infected patients significantly affects myeloid and lymphocytoid lineages and increases the risk of invasive Aspergillosis with a severe prognosis. Although Aspergillus prophylaxis is not routinely indicated in HIV patients it should be considered in cases of prolonged neutropenia. Rapid evolutive Aspergillosis is rare in the presentation of Hodgkin's lymphoma but should nevertheless be included in the differential diagnosis.

Keywords: Aspergillus fumigatus, lymphoma, HIV

Acute prolonged tonsillitis with fungal etiology after chickenpox

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Background: In conditions of low immunity, some commensal germs of the oropharynx, in this case *Streptococcus oralis* and *Candida albicans*, may become pathogens, causing tonsilitis with drawling progression[1].

Materials and methods: 4-years-old patient in convalescence after varicella has presented for 10 days of fever (40.1°C), chills, bilateral laterocervical adenopathy, dry cough. He was successively treated with Clarithromycin, Ceftriaxone and Gentamicin at indication of his family doctor. Symptomatology persists, and the pacient was admitted inin Infectious Diseases Clinic. Clinical examination revealed: general influenced state, low grade fever, pale skin, matte tongue, hypertrophic tonsils with whitish deposits. Pulmonary auscultation: without rales/crackles. Biological and paraclinical investigations were performed to establish the positive diagnosis.

Results and discussion: Leukocytes 35 430/μL, neutrophils 80%, lymphocytes 9.7%, monocytes 10.1%, eosinophils 0.1%, VSH 90 mm/h, CRP 122.64mg/L, ALT 12.3 U/L, AST 14.5 U/L. Blood smear: leukocytosis, neutrophilia.. Non-reactive IgM-CMV, RFC *Mycoplasma pneumoniae* negative, RFC-Adenovirus: 1/8. Pharyngeal exudate: Culture for bacterial flora - present *Streptococcus oralis*; belonging to normal oral flora, it could not be incriminated in this membranous tonsillitis. Fungal culture *Candida albicans* >100 UFC. Blood culture: No bacterial growth. Chest radiography: No alveolar condensation or pleural effusions. Direct hypopharyngoscopy: Quantifiable and detachable rich caseus deposits highlighted at the base of the tongue. Sample of caseum was taken for culture. He initially received antistreptococcus treatment (accordingly to literature, Streptoccocus spp. are the main ethiology in acute tonsillitis[2]), with Penicillin G 2x600,000 UI for 12 days + Oxacillin 4x500 mg/day for 4 days, then was Fluconazole 200 mg//day. . Evolution was favorable but slowly till Fluconazole addition, then he recovered. The patient was discharged with indications of vitaminotherapy at home. *Candida albicans* is known as a commensal germ, but with those with immunosuppressed conditions may become an intermittent pathogen[1].

Conclusions: Immunodepressed patients in convalescence of viral diseases such as varicella can develop prolongued tonsilitis with double etiology, bacterial and fungal.

Keywords: tonsilitis, *Candida albicans,* , varicella

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Aspergilloma in an adult with post-tuberculosis sequelae

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Background: Fungi of the genus *Aspergillus* are spread ubiquitously in the environment, and they can be isolated from soil, plants, organic matter in decomposition, water, food. Most commonly, infections with these species, affect the lung, especially in immunocompromised individuals (1,2).

Materials and Methods: An 80-year-old patient, with a bacillary history, is presented on the department accusing: persistent non-productive cough with hemoptysis, weight loss (10 kg), severe asthenia, chills. Objective clinical examination: general state moderately altered, TA 160/90 mm Hg, HR 120 bpm, rhythmic, hypotrophy (IMC 19.75 kg/m^2), deformed thorax; pulmonary auscultation: without rales, SaO_2=95%. Biological samples and imagery required for diagnosis were performed.

Results: Hemoglobin 9.8 g/dL, Hematocrit 30.9%, Neutrophil 67%, Lymphocytes 20%, CRP 28.96 mg/L, ESR 40 mm/1h, D-dimers 374.7 ng/ml, negative Gram smears bacteria and GeneXpert negative for M. tuberculosis. Chest radiography: cavitary image with thick, apical left-handed walls. Native thoracic CT scan revealed microcalcifications and bronchiectasis in apical part of left superior lobe, in which there was an oval round body with irregular walls - possibly mycetoma and adjacent pleural thickening. There were also linear postero-basal bilateral fibrous tracts, and three other nodular lesions in right lobe, possible sequelae after tuberculosis Thoracic surgery consult suggested a mycetoma on preexistent apical cavern. Treatment with hemostatic and antifungal agents (Itraconazole 400 mg/day) was started, with favorable evolution with diminished symptomatology and remission of hemoptysis. The main clinical forms of Aspergilloma post-tuberculosis are: simple aspergilloma, chronic cavitary pulmonary aspergillosis and chronic fibrosing pulmonary aspergillosis[1]. Chronic pulmonary aspergillosis is commonly associated with fatigue, haemoptysis, weight loss, and breathlessness. Antifungal therapy contributes to ameliorating symptoms and reducing recurrence of haemoptysis[2,3]. Untreated pulmonary aspergillosis may contribute to increased of mortality in patients with post pulmonary tuberculosis syndrome[1].

Conclusions: Pulmonary aspergilloma is one of treatable causes of hemoptysis and could appear in preexistent cavitary lesions, as those of tuberculous sequelae.

Keywords: aspergilloma, pulmonary tuberculosis cavernous sequelae, hemoptysis

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Pulmonary aspergillosis due to *Aspergillus niger*: case report

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Background. *Aspergillus* is a fungus that usually lives in soil, but can be found also in food, and indoor and outdoor air [1,2] The spores are airborne and become easily inhaled. In the respiratory tract, the spores can germinate into hypes that can invade the mucosa leading to invasive pulmonary aspergillosis. The immune answer of the host and the inflammatory cells can limit the fungal growth and can prevent the disease in the majority of cases [3].

Materials and methods. We present the case of a patient with pulmonary tuberculosis in antecedents and ankilopoetic spondylitis, hospitalized for cough with haemoptoic and mucopurulent expectoration, persistent fever, dyspnea and alteration of the general state. Initial laboratory tests revealed neutrophilic leukocytosis and *Aspergillus niger* in the tracheal aspirate. The CT scan shows a lung cavity containing hyperdense "sponge-like" tissue and multiple inhomogeneous areas of consolidation with a tendency to form abscesses. The treatment with Posaconazole, Amikacin, Cefotaxime and Metronidazole was initiated.

Results and discussions. Despite the treatment, the febrile syndrome persisted. In these circumstances, the therapy was replaced by itraconazole, with a temporary remission of the fever. Further laboratory tests revealed in the sputum the presence of *Streptococcus pneumoniae*, so the antibiotic treatment was changed, based on susceptibility testing results, with Ampicillin and Trimethoprim/Sulfamethoxazole. The recurrence of febrile syndrome determined a new reshuffle of the antifungal medication, with voriconazole. This time the therapeutic answer was favorable. On hospitalization, the patient presented a positive stool test for *Campylobacter* and *Clostridium difficile* infection.

Conclusion. Fungal infection has occurred on an immunosuppressed status in the presence of other risk factors such as the remaining TB cavity, and this fact led to the necessity for successive therapeutic reshuffles. This case illustrates the therapeutic difficulties that may arise in the situation of multiple pathological associations.

Keywords: aspergillosis, treatment, difficulties

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Candiduria in inpatients from a Turkish tertiary hospital between 2006-2016

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Background. Urinary tract infections (UTIs) caused by *Candida* species are increasing rapidly due to surgical and medical applications especially in hospitalized and intensive care unit (ICU) patients. Although *Candida albicans* is the most common species isolated from urine samples, there is growing evidence of shifts to more resistant strains (1). Therefore, isolation and identification of fungi causing UTIs are extremely important for their appropriate treatment (2). Therefore, we evaluated the distribution of funguria agents according to years and sources in our tertiary care hospital for 11-year period.

Materials and methods. We retrospectively analyzed urine culture results obtained from Microbiology Laboratory in our University Hospital during a 11-year period from January 2006 to January 2017. The results including pure yeast growths accompanied by pyuria in inpatients were included in this analysis. Antifungal susceptibility of these isolates against fluconazole and voriconazole were evaluated by disk diffusion method (CLSI M44-A) (3). Repetitive results of the same patients were excluded. All results were classified according to both year and hospital departments.

Results and discussions. Approximately 36500 positive urine culture results were evaluated. The rate of yeasts in all isolates was 9.7% (n=3540) and 3328 of them were from inpatients. When the inpatients were evaluated according to hospital departments, the rates changed conspicuously; from 16% to 30.7% in internal medicine services and ICU, from 7.6% to 24.7% in pediatric services and ICU, from 9.1% to 38.4% in surgical services and ICU. Generally, *C. albicans* was the most common isolated species (54.2%), followed by *C. glabrata* (15.5%) and the rate of non-*Candida* yeasts was 3.5%. However, while the frequency of *C. albicans* decreased, the frequency of non-*albicans Candida* and other yeasts exhibited an increase in years. Resistance to fluconazole was observed for three *C.albicans* (0.16%), eighty *C.glabrata* (15.5%), seven *C.tropicalis* (2.1%) and all *C. krusei* isolates. Voriconazole resistance was lower; fourteen *C.glabrata* (2.7%), one *C.krusei* (0.8%), two *C.tropicalis* (0.6%) and fluconazole resistant *C.albicans* (0.16%) isolates.

Conclusions. Although, the agents of UTIs are frequently bacteria, yeasts - especially *Candida* spp.- are important pathogens for the patients in ICUs. The most common species is *C.albicans*, but the frequency of non-*albicans Candida* species and other yeasts are increasing. Therefore, microbiological diagnosis, identification and susceptibility testing should not be neglected.

Keywords: Candida, urine culture, frequency

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Involvement of *Candida* species in the etiology of some human dermatomycoses diagnosed in outpatients

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Aim. Fungal infections of the skin, hair and nails are a common public health problem worldwide (1,2). Their incidence is closely related to the associated pathology, both adults and children being exposed to these dermatomycoses (3) . Our study aims to determine the incidence and distribution of *Candida* species in the pathology of superficial dermatological infections.

Materials and methods. A number of 93 isolates were recovered from patients with suspicion of dermatomycosis. Samples were collected in sterile containers by scraping a portion of the epidermis or nail and by pulling out with a tweezer the affected hair. The biological products were cultivated on Sabouraud's medium supplemented with chloramphenicol and gentamicin and on Mycosel Agar (Micobiotic Agar) respectively. Incubation was performed at 30^0C for a maximum period of 30 days. Positive cultures were subsequently processed and the yeast isolates were identified by mass spectrometry using MALDI-Biotyper (Bruker).

Results and discussion. The study revealed a 5% positivity with a female/male *ratio* of 70%:30%. Depending on the harvested product, a dominant *ratio* of nail (75%) was observed compared to squamous tissue (25%) infections. Distribution by age group revealed the predominance of dermatomycoses in adult (84 isolates) compared with the pediatric population (9 isolates). The percentage distribution of *Candida* species was different by the age group. Thus, *C. parapsilosis* (45%), *C. albicans* (22%) *C. lusitaniae* (22%) and *C. zeylanoides* (11%) predominated in children (average age of 5 years). For the category of adult population (average age of 43 years), *C. parapsilosis* (45%), *C. albicans* (29%), *C. guilliermondii* (14%) and *C. lusitaniae* (4%), *C. metapsilosis* (3%) prevailed, followed by *C.intermedia* and *C. orthopsilosis* (2%) and *C. tropicalis* (1%).

Conclusions. Laboratory diagnosis of human dermatomycosis has a key role in determining the microbial etiology, allowing the identification of the genus and species of the fungal agent. In both age groups the predominant species were *C. parapsilosis* and *C. albicans.*

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Fulminant cryptococcal meningoencephalitis following reactivation of primary cutaneous cryptococcosis

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Background: Encapsulated fungi, such as *Cryptococcus neoformans*, may cause disease both in the immunocompromised as well as the immunocompetent host. Primary infection is the mainstream epidemiologic event; however, the fungus may become retained in latency, usually through colonization of the respiratory system, and cause disseminated disease when immune function becomes compromised.

Case report: Herewith we present a rare case of a 66-year old oncology patient developed fulminant cryptococcal meningoencephalitis two years after a scalp skin infection secondary to an olive tree branch trauma. At the time of diagnosis, the patient had initially received oral fluconazole and subsequently intravenous liposomal amphotericin B due to the development of resistance for cutaneous cryptococcal skin infection. Two years later, in the course of chemotherapy for newly diagnosed gastric and lung cancer, the patient developed fever and neurological symptoms and was diagnosed with fulminant cryptococcal meningoencephalitis. Based on the patient's history treatment was initiated with intravenous liposomal amphotericin B (4 mg/kg) and IV flucytosine (25 mg/kg every 6h). HIV antibodies tested negative and immunophenotype of peripheral blood cells revealed normal CD4/CD8 cell ratio and an increased number of NK cells. The patient's clinical condition rapidly deteriorated (loss of vision and intense neck rigidity). Repeated magnetic resonance imaging of the brain revealed lesions compatible with infectious meningoencephalitis. This is a rare case of fulminant cryptococcal meningoencephalitis following an adequately treated primary cutaneous infection. Latency state in the infected host is a well-recognized mechanism of disease pathogenesis of these pathogenic fungi, and accounts for disseminated disease in the immunocompromised patient. **Conclusions**: To our knowledge, this is the first reported case of cryptococcal dissemination following primary cutaneous cryptococcosis.

Keywords: cryptococcosis; meningoencephalitis

A case of fatal sepsis with double etiology in an HIV infected patient

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Background: Cryptococcosis is a deadly opportunistic infection caused by Cryptococcus neoformans, an encapsulated yeast that is present in soil contaminated with pigeon excreta and it is distributed all over the world. The organism enters the body through respiratory tract and dissemination is haematogenous to CNS, skin, bone, lymph node, kidney, liver, spleen and other viscera. Cryptococcal meningitis with disseminated cryptococcosis is one of the most common life-threatening fungal infections in AIDS patients. It is invariably fatal if left untreated, and carries a high mortality even with treatment.

Material and methods: The authors present a clinical case of disseminated cryptococcosis in a patient with advanced HIV disease. The patient was diagnosed with HIV infection in June 2015, as a very late presenter (CD4=14 cells/µl, HIV- ARN=175.357 copies/ml) with persistent fever, oral and esophageal candidiasis, generalized pustulosis and wasting syndrome. The imagistic evaluation (abdominal echography and CT scan) had shown multiple splenic abscesses. The CSF direct examination and cultures were positive for *Cryptococcus neoformans*.

Results and discussions: Under conservatory treatment, including Fluconazole high doses, the evolution was unfavorable. The patient needed splenectomy. The splenic tissue cultures revealed *Cryptococcus neoformans* and, surprisingly, *Acinetobacter baumannii.* We did not perform an echocardiography so we could not rule out an endocarditis explaining splenic abscesses.

Despite the administration of antifungals, antibiotics and antiretroviral treatment, the evolution was unfavorable leading to death. In HIV infected patients, disseminated cryptococcosis, as in this patient, is due to defects in T-cell function and may represent a primary infection or reactivation of latent infection acquired many years earlier. The clinical presentation of disseminated cryptococcosis is variable and depends on the organ and systems involved. Although the most common form of the disease is meningitis, dissemination to extraneural sites can be seen and usually carry grave prognosis. In this case, the severity was amplified by the presence of the second agent – *A. baumanii*, which proved also to be disseminated, being revealed in splenic abscesses.

Conclusions: The high mortality rate of HIV-related cryptococcal disease is due to the inadequacy of current antifungal therapy, restricted access to drugs of first choice in many areas including Romania and the problem of raised CSF pressure. Even with optimum treatment the mortality remains high. The second agent - *A. baumanii*, that is not usually involved in infections in HIV-positive patients, contributed also to the fatal evolution.

Keywords: *Cryptococcus neoformans*, *Acinetobacter baumanii*, Acquired immunodeficiency syndrome (AIDS), splenic abscesses

Fungal detection by commercial multiplex real-time PCR (LightCycler® Septi*Fast*) in Intensive Care Units (ICUs) hospitalized patients with suspected sepsis: a retrospective study from Greek hospitals (2010-2017)

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Background: The study presents the impact of a commercial available multiplex PCR system in the diagnosis of fungal infections among patients with suspected sepsis during their hospitalization in Intensive Care Units (ICUs). Although blood culture (BC) is considered the standard criterion for diagnosis of bloodstream infections (BSI), it takes time for final identification.

Materials and methods: Blood samples from patients with presumed sepsis were cultured with conventional automated blood culture systems [Bactec 9240™ system (Becton Dickinson) or BactAlert system (BioMerieux)] and blood in EDTA from the same patients subjected to analysis with a commercial multiplex real-time PCR (LightCycler® Septi*Fast* assay, Roche Molecular Systems). LightCycler® Septi*Fast* (SF) assay uses a renovated technology that enables the direct detection of the commonly involved pathogens in systemic infections, through a wide panel of Gram-negative, Gram-positive and fungal pathogens (5 *Candida* species and *Aspergillus fumigatus*).

Results and discussions: During the period 2010-2017, 697 SF tests were collected from 534 hospitalized patients. In 517 patients (97%) the SF test was performed with ongoing empirical antimicrobial therapy. Fungal etiological definition was achieved in 24 BSI episodes of the total 148 positive results (i.e. 16%). The fungal pathogens in the 24 positive cases are shown in Table:

Fungal pathogens	Detected cases	Detected cases by method	
		BC(+)	LC-SF(+)
C. albicans	**12**	**10**	**12**
C. parapsilosis	**6**	**2**	**6**
C. tropicalis	**2**	**2**	**2**
C. krusei	**1**	**-**	**1**
C. albicans/tropicalis	**1**	***C. tropicalis***	***C. albicans / C. tropicalis***
Aspergillus fumigatus	**2**	**2**	**2**

Both SF and BC identified the responsible fungal pathogen in 16 from 24 cases. The SF test reduced the time of diagnosis with a mean of 14 h, in contrast to the 48-72 h required for blood culture. Also, the positive results of *A. fumigatus* in SF assay were confirmed with galactomannan antigen serum test. According to the SF results, initial therapy was inadequate in 17 patients, and antifungal treatment was added promptly in all patients with fungemia.

Conclusions: The rapid multi-pathogen PCR (SF) system can be used as a diagnostic tool for the timely detection of fungi, having a relevant impact on targeted antifungal treatment.

Key words: Fungal infections, ICU, Septi*Fast* test, sepsis

Comparative study of three methods for the preservation of yeast isolates

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Background. Various methods for the preservation of yeast isolates are described in medical literature, concerning temperature, material and duration of preservation (1,2,3). The aim of this study was to compare the efficacy of three methods, checking if the viability of the isolates depends on temperature or duration of preservation, in order to choose an easy, stable and reliable method.

Materials and methods. The study included 45 yeast isolates preserved using three different methods. Isolates of Group 1 and 2 were preserved in cryovials containing sterile distilled water at room temperature and 4°C respectively, while the isolates of Group 3 were preserved in aqueous solution of 15% glycerol at -20°C. Duration of preservation varied from 12 to 27 months. All isolates were subcultured at 35°C on Sabouraud Dextrose Agar with chloramphenicol 0.05% and plates were inspected at 24, 48 and 96 hours after inoculation. Statistical analysis was performed by the means of x^2 and Spearman r tests.

Results and discussion. The total survival of the isolates was 84.4% (38/45) for Group 1, 82.2% (37/45) for Group 2 and 88.8% (40/45) for Group 3. Concerning survival over the method of preservation there was not found any statistically significant difference (p=0.663). There was not found any statistically significant difference in the group of preservation for 12-19 months or the group of 20-27 months (p values 0.348 and 0.418 respectively). There was not found any correlation between the duration of the storage and the possible loss of the isolate. *Candida albicans* isolates were compared to non-*albicans* C*andida*, and the later demonstrated better overall survival (p=0.019), due to better survival at 4°C (p=0.021) and especially at the longer preservation of 20-27 months (p=0.002).

Conclusions. Overall survival of yeast isolates was not affected by temperature or duration of preservation. Non-*albicans* C*andida* isolates exhibited a better survival than *Candida albicans* at 4°C, especially when preserved from 20-27 months. Each laboratory may follow any method of the three but due to practical or financial reasons, material availability and probable not constant conditions of room temperature, the preservation at 4°C seems to be the more reasonable approach.

Keywords: *Candida albicans,* non-*albicans* C*andida,* yeasts, preservation methods, temperature.

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Preliminary in vitro comparison between two commercial kits for the detection of 1-3-β-D-Glucan in serum.

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Background. 1-3-β-D-Glucan is a fungal cell wall component that has shown promising results as a screening tool in the diagnosis of several invasive mycoses. A sensitivity and specificity range of 55-95% and 77-96% respectively have been referred, while it is included in the EORTC diagnostic criteria.

Several commercial kits have been released and the aim of this study was to compare the recently available kit of Dynamiker Fungus (1-3)-β-D-Glucan assay (Dynamiker Biotechnology Co, Ltd, China) to the broadly used, evaluated and FDA cleared Fungitell® assay (Associates of Cape Cod, USA).

Materials and Methods. Twenty-four serum samples from equal number of patients with clinical suspicion of invasive mycosis were tested. All specimens were collected under glucan free conditions. In ten cases, there was a positive galactomannan measurement (Platelia™ Aspergillus Ag, Bio-Rad, France). Both glucan kits are based on the same principle and the measurement is performed under kinetic conditions in 37°C. The Dynamiker method provides two more vials of the main reagent (although the total volume is almost the same) and the standard solution. It is performed using eight-well breakable strips instead of the whole 96 well micro-plate of Cape-Cod. The first method uses 20 µl of serum and its positivity threshold is set at 95 pg/ml, while the second uses 5 µl and its threshold is at 80 pg/ml. Both methods have a similar standard curve range (37.5-600 pg/ml and 31-500 pg/ml respectively).

Results. The overall agreement on clinical level (positive or negative) between the two methods was 67% (16/24). Values didn't differ statistically ($p=0.15$) while they were moderately correlated ($r=0.42$, $p=0.039$). If the Dynamiker method was compared according to Fungitell positive results, then the agreement was 33% (4/12), while according to negative Fungitell it was 100% (12/12). Concerning the ten serum samples with positive galactomannan, the agreement was 50% (5/10), 9/10 were found positive by the Fungitell, 4/10 by the Dynamiker, while both methods were negative in one case of positive galactomannan. In two cases of bacteraemia solely the Fungitell provided a positive result. Considering as a criterion the positive or negative galactomannan for the diagnosis of aspergillosis, the comparison proved a diagnostic accuracy of 87% for Fungitell and 74% for Dynamiker.

Conclusion. Although this study included only a limited number of clinical specimens the results showed that the Dynamiker method, being highly specific, could be a useful alternative after further in vitro and clinical evaluation.

Species distribution and antifungal susceptibility profile of *Candida* isolates from intensive care patients: a five-year study

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Background. The incidence of nosocomial *Candida* infections has increased in recent years due to an increased number of patients receiving chemotherapy and other immunosuppressive therapies or undergoing organ transplantation, the use of broad spectrum antibiotics, the increased number of patients in intensive care units, and invasive procedures in patients.[1,2] *Candida* species are isolated as the fourth most common pathogen causing nosocomial bloodstream infections and *Candida albicans (C.albicans)* is the most common cause of nosocomial infections.[3,4]

Retrospectively, our study aimed to determine the species distribution and antifungal susceptibility of *Candida* strains isolated from intensive care blood cultures during the last five years in our hospital.

Materials and Methods. *Candida* isolates from blood cultures sent from our intensive care unit between January 2013 and December 2017 were evaluated. Blood samples were placed in Bactec Ped Plus for pediatric patients and Bactec-Plus aerobic bottles (Becton-Dickinson, USA) for adults, transferred in the BACTEC 9120 automated system and incubated for five days in that device. Fungal isolates were identified at species level using conventional methods and API ID 32C (bioMerieux, France). Susceptibility of *Candida* isolates to amphotericin B, fluconazole, caspofungin, ketoconazole, voriconazole and itraconazole were determined using the E-test (bioMerieux) gradient method. Minimum Inhibitor Concentration (MIC) values were determined.[5] *C. albicans* ATCC 10231 was used as a control strain.

Results and Discussions. During the 5-year period, 79 out of 3978 patients had at least one positive blood culture for yeasts. Forty-three patients were male and 36 were female. The species distribution was as follows: *C. parapsilosis* n=37 (47%), *C. albicans* n=27 (34%), *C. glabrata* n=6 (7%), *C. kefyr* n=2 (2%), *C. lusitaniae n*=2 (2%), *C. neoformans* n=2 (2%) and *S. cerevisae* n=2 (2%). *Candida parapsilosis* was the most common, and *Candida albicans* was the second one. All isolates were susceptible to caspofungin. For the other antifungals, the percentages of susceptible strains are: Amphotericin B 94%, Fluconazole 34%, Itraconazole 60%, Voriconazole 38%, Anidulafungin 50%, Ketoconazole 21%.

Conclusions. Candida-associated bloodstream infections represents 8-10% of all nosocomial bloodstream infections and 10-20% of all nosocomial bloodstream infections in intensive care units (6,7). *Candida* species in normal body flora cause infection by passing natural barriers through the application of invasive procedures such as catheters and endotracheal tubes in intensive care patients (8). It is stated that *C. albicans* causes more endogenous infections, *C. tropicalis* and *C. parapsilosis* can be nosocomial transmitted, and infections with these two types can be seen more frequently if hospital infection control measures are not followed.[9]

Keywords. Bloodstream infections, Candida species, intensive care patients, antifungals

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Screening for yeast species colonizing the orofarynx and dorsal tongue surface in dental students

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Background: The normal flora of the oral cavity (especially the dorsal tongue surface) and oropharynx includes yeasts in small percent, with *Candida albicans* being the predominating ones. The purpose of this study was to investigate the yeast species which colonize the oropharynx and dorsal tongue surface in healthy dental students.

Materials and methods: Oropharyngeal swabs (OFS) and tongue swabs (TS) collected by rubbing against the dorsal tongue surface were obtained from 83 healthy dental students in the second academic year at the Faculty of Dental Medicine (FD), "Carol Davila" U.M.F. (UMFCD) - Bucharest, in the first trimester of 2018. The samples were seeded on Sabouraud agar with gentamicin and chloramphenicol (BioMérieux, France). The isolates among the yeast strain collection obtained from this student series were identified based on the colony color on *Brilliance* Candida agar (Oxoid, UK), result of the germ tube test and ID 32 C system (BioMérieux, France). In addition, the susceptibility of the *Candida* isolates was tested against 5 antifungal agents by diffusion method with strips with predefined gradient of antifungal drug concentrations and ATB FUNGUS 3 system (BioMérieux, France).

Results and discussions: Fungal strains were isolated from 3 OFS and 10 TS samples. Ten strains produced green colonies on the chromogenic agar, showed positive result for germ tube test and were identified as *C. albicans* by the ID 32 C system. Three strains originated from TS samples were identified as: *C. parapsilosis*, *C. famata* and *Rhodotorula mucilacinosa*. All *Candida* strains tested against the 5 antifungal drugs were susceptible except for one *C. albicans* isolate, which showed resistance to fluconazole.

Conclusions: In this dental student series, the rate of yeast carriage on tongue surface and oropharyngeal site was of 12% and 3.6%, respectively. As expected, *Candida albicans* predominated among the 4 fungal species identified during the study. Screening for the susceptibility of *Candida* isolates in healthy subjects may be useful for finding possible reservoirs of antifungal resistant yeasts at oral sites.

Acknowledgements: This study is part of the internal research plan of the Microbiology Department of FD, in collaboration with a member of the Epidemiology Department, UMFCD – Bucharest.

Keywords: yeast carriage, oropharynx, dorsal tongue surface

Species distribution of *Candida* isolates colonizing patients admitted in an Intensive Care Unit from Giannitsa-Greece

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Background. In the past years there has been observed an increase in the incidence of the hospital fungal infections which can put even the lives of the patients in danger. The patients mostly in danger are the ones of the Unit of Intensive Care (UIC). The colonizing rhythm of *Candida* spp. reaches up to 80% to patients lying in the units of intensive therapy more than seven days and the average developing rhythm of the filtering disease is 10% to the colonizing patients. Consequently, the colonizing control has been globally established because the rapid diagnosis of *Candida* spp. and treatment are both important, as discovered from the percentages above.

Materials and Methods. During the year 2017 we recorded the frequency of the isolating types of *Candida*, from colonizing cultures of patients in the Unit of Intensive Care (UIC), which have been hospitalized in the General Hospital of Giannitsa. In 232 culture samples taken from the nose and the pharyhx mucuous membrane, the bronchial lavage, the armpit surfaces, rectum and urine of 108 patients (60 males, aged 66 to 88, and 48 females, aged 61 to 92) different species of *Candida* were isolated (1,2). The identification was made using the morphology of the colonies on Chromagar Brilliance (4,5).

Results and discussions. Out of the 232 cultures of the 108 patients in the whole, the following species were indentified according to the rate of frequency: *Candida albicans* 59%**,** *Candida glabrata* 20%**,** *Candida tropicalis* 15%**,** *Candida krusei* 7%.

Conclusions. All the cultures of patients exhibited *Candida* yeasts, the prominent type was *C. albicans* with the rest of the types showing also an increase in frequency that is a significant observation of the past years. These non-albicans *Candida* species play a crucial role in the therapy outcome of the occasional infections occurring in these vulnerable patients (3).

Keywords: *Candida*, Intensive Care Unit , colonizing yeast

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Genotypic analysis of candidaemias occurring in two Greek hospitals using microsatellites

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Background. *C. parapsilosis* is notorious for its association with catheters, hospital equipment, or the colonization of the hands of the nursing staff. In order to study nosocomial cross-transmission, molecular typing of *C. parapsilosis* outbreak isolates is of utmost importance. Microsatellites are believed to be suitable markers in that respect. The scope of this study was to assess the epidemiological relatedness of *C. parapsilosis* strains isolated from candidaemias occurring in two tertiary care Greek hospitals.

Materials and methods. The strains were derived from the hospitals Tzaneio General Hospital of Piraeus, (hospital 1) mostly from the ICU, and Laiko General Hospital of Athens (hospital 2), mostly from the Surgical and Medical Units, during the years 2007-2013. In total, 50 strains were studied; 24 clinical isolates from hospital 1; 22 clinical isolates from hospital 2; 4 non-clinical strains, 3 isolated from the hands of nursing staff and one from a medical trolley cart, all from the ICU at hospital 1. All strains were confirmed to be *C. parapsilosis sensu stricto*. For microsatellite genotyping, a panel of six short tandem repeat (STR) markers was used; 3A, 3B, 3C, 6A, 6B, 6C. Three trinucleotide repeat markers were amplified in a multiplex PCR and analyzed in an ABI3500xL fragment analyzer (Applied Biosystems™, MA USA).

Results and discussions. Analysis revealed 29 distinct genotypes in total. Of the isolates from hospital 1, 18/28 (64.3%) were clustered in 4 genotypes, 2-9 strains each, including a strain isolated from a nurse's hands. Of the isolates from hospital 2, 7 (31.8%) were clustered in two genotypes, 3 and 4 strains each. In both hospitals, strains isolated in different years appeared in the same cluster, but genotypes of the two hospitals were distinct.

Conclusions. Our results indicate that certain *C. parapsilosis* strains may reside locally and persist in health care facilities, causing occasional outbreaks. We further show that in hospital 1, there was an outbreak of four candidaemia cases in 2013, involving a strain isolated from the hands of nursing staff.

Keywords. *Candida parapsilosis*, epidemiology, genotyping, microsatellites

Frequency of superficial fungal infections in a primary healthcare unit in Greece

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Background. Superficial fungal infections are common worldwide and their frequency continues to grow. The causal agents are dermatophyte, non-dermatophyte filamentous fungi and yeasts. They don't consider to be life threaten in non-immunosuppressed patients but causes unpleasant symptoms such as pain, inflammation and long-time therapy (1, 2).

Materials and Methods. A survey for superficial fungal infections conducted during January 2015 to December 2017 at a Primary Health Care Unit in Thessaloniki, Greece. A total number of 209 patients (66 men and 143 women) were investigated and the collected specimens distributed as follow: 29 cases of skin, 25 of hair and 157 of nails (90% of which were toenails). All specimens subjected to direct microscopy examination in 10% potassium hydroxidate (KOH) and fungal culture on Sabouraud's Dextrose Agar with chloramphenicol and kept at 30^0 C for 1 month. Identification of fungi was made by microscopical and macroscopical observation of the grown colonies (3).

Results and Discussions. Ninety-five out of 209 fungal cultures were positive (45%), a quarter of which considered without clinical significance (saprophytes). Among of culture proven infections, the frequency of dermatophytes was 52.9% with *Trichophyton* spp. being the most common isolate (91.9%) in contrast to *Microsporum* spp. (8.1%). The frequency of yeasts was 18.6% and of non-dermatophytes filamentous fungi was 28.6% (45% identified as *Aspergillus*, 35% as *Fusarium* and 20% as other species, such as *Acremonium*, *Alternaria* etc.).

Table 1. Frequency of fungal infection per collected specimen

Toenail	Fingernail	Skin	Hair
Trichophyton spp. 49%	*Trichophyton* spp. 25%	*Trichophyton* spp. 75%	*Trichophyton* spp. 33%
NDM 39%	Yeast 75%	Yeast 12,5%	*Microsporum* spp. 67%
Yeast 12%		*Microsporum* spp. 12,5 %	

Conclusions: Dermatophytosis is the most frequent form of superficial fungal infection and the most common isolate is *Trichophyton* spp. Regarding nail infection the most frequent causal agent in toes is *Trichophyton* spp., while in fingers is *Candida* spp. *Trichophyton* spp. was mostly isolated from skin infections and *Microsporum* spp. from hair. Our findings are in accordance with previous studies conducted in Greece.

Keywords: superficial fungal infections, primary health care unit, dermatophytosis

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Pneumocystis pneumonia - a retrospective study during 2009-2018 in the National Institute of Infectious Diseases "Matei Bals", Bucharest, Romania

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Background. *Pneumocystis jirovecii* pneumonia (PCP) is induced by a human-specific ascomycetous fungus commonly found in patients with severe immunosuppression (1–3). Currently there is no national surveillance on PCP and associated epidemiologic data is scarce (4). The aim of this paper is to provide a retrospective study on PCR cases admitted to a tertiary hospital in Romania and to compare the epidemiologic differences between two-time period, namely 2009-2013 and 2014-2018

Materials and Methods. The study was performed on 178 HIV-infected patients admitted with PCP between January 2009 and May 2018. Finally, only 88 (49.43%) patients fulfilled clinical, radiologic and laboratory data suggestive of PCP, namely sub-acute onset of cough, dyspnoea and/or fever, ground-glass or bilateral perihilar interstitial infiltrates on chest X-ray or thoracic computed tomography and/or microbiologic confirmation on bronchoalveolar lavage through microscopy or real-time PCR (5). Statistical analysis employed non-parametrical chi-square and Mann Whitney tests and Pearson correlations, with p values below 0.05 as statistically significant.

Results. Of the 88 (49.43%) patients with suspected PCP, only 20 (22.7%) were confirmed on smear exams or PCR. PCP was revealing for the HIV diagnosis in 42 (53.4%) of patients. Conversely, only one third of previously diagnosed inviduals 33 (37.5%) were following antiretroviral treament and even fewer 5 (15.2%) were adherent to treatment. We recorded 36 (40.6%) deaths despite rapid treatment. The analysis of associated infections revealed frequent fungal co-infections (66, 76.13%) and fewer viral and bacterial co-infections (25, 28.4% and 19, 21.5% respectively). Comparative analysis between 2009-2013 and 2014-2018 revealed a higher number of confirmed cases (12.8% versus 34%, p value=0.017) and fewer deaths (51.1% versus 29.3%, p value = 0.038) in the latter period. Additionally, PCP survivors displayed significantly lower values of serum LDH (p value <0.001) with higher albumin, CD4 T cell counts and CD8 T cell counts (p value= 0.049 and respectively 0.032, 0.020).

Conclusion. PCP remains a diagnostic and therapeutic challenge, particularly in young patients. While roughly one half of PCP cases continue to involve HIV late presents, the study shows that PCP is currently more easily confirmed and is associated with fewer deaths.

Keywords: Pneumocystis pneumonia, HIV

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ETIOLOGICAL AGENTS OF DERMATOMYCOSES IN MACEDONIAN PATIENTS

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Background: Dermatomycoses are among the most common skin diseases that are a major public health problem. This study aimed to prospectively determine the etiological agents of dermatomycoses, in a group of dermatology patients treated in dermatology outpatient departments, which could contribute to better clinical outcome and better control of these infections.

Material and methods: This was a prospective study which was carried out over a period of 3 years. During this period, a total of 127 specimens (91 nail specimens, 27 skin scrapings and 9 hair specimens) from patients with clinically suspected dermatomycoses, who attended the dermatology outpatient departments of both City General hospital and University clinic of Dermatology in Skopje, Macedonia, were examined. These specimens were evaluated with conventional mycological procedures (culture and direct microscopy). They were inoculated on a standard mycological media (selective medium for fungal growth) and incubated up to 3 weeks on room temperature and 35°C. Identification of the etiological agents was based on the colony pigmentation, texture and microscopic features of the fungi (macroconidia, microconidia with lactophenol blue direct investigations).

Results: Thirty three percent (42/127) of the specimens were found to be culture positive. Males were infected more (26/42) than females (16/42). The commonest age group was 41-50 years. The most frequent etiological agents were non-dermatophyte molds-73.8% (31/42), yeasts-16.7% (7/42) and dermatophytes-9.5% (4/42). Seventy four percent of the positive findings originated from nail specimens (31/42). The agents of onychomycosis, in order of frequency, were: *Cladosporium* sp. (7), *Aspergillus flavus* (5), *Aspergillus fumigatus* (3), *Fusarium* species (3), *Paecilomyces* species (2), *Alternarira* species (2), *Scopulariopsis* species (2) unidentified *Aspergillus* species (2), *Phoma* species (1). *Candida albicans* was identified in 19% (8/42) cases of onychomycosis. The fungi recovered from skin scrapings were: *Trychophyton* species (2), *Trichosporon* species (1), *Curvularia* species (1) and *Onychocola* species (1). *Microsporum* species was identified in 2 hair specimens.

Conclusion: This study shows that non-dermatophytic molds were responsible for more than sixty percent of dermatomycoses' cases. Since molds can be common contaminants of the specimens, consecutively taken specimens should be investigated and carefully evaluated in order to diagnose a "mold dermatomycoses".

Key words: dermatomycoses, molds, yeasts, dermatophytes

Epidemiologic trends of pediatric candidemia in a tertiary care institution over a 12-year period

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Background: Candida species are the leading cause of invasive fungal infections in hospitalized children and are the third most common isolates recovered from patients with healthcare-associated bloodstream infection [1,2,3].To record the changes in the epidemiology of candidemia in the pediatric patient population of a tertiary hospital over a 12-year period.

Materials and Methods: All episodes of candidemia that occurred in children (excluding neonates) during a 12-year period since 2001 were captured in the microbiology laboratory database. *Candida* species and department of origin (PICU, two general pediatric wards, pediatric surgery and pediatric oncology wards) were recorded. In addition, case outcome was also recorded. Statistical analysis of incidences between early and late periods and among different departments as well as among different frequencies was performed by chi-square test.

Results and discussions: In this 12-year period, 105,558 pediatric patients were admitted in the hospital and 41 episodes of candidemia were recorded accounting for a 3.88 cases per 10,000 admissions (IQR: 4.41). PICU patients comprised 46% (19/41) of the total episodes, oncology patients 12.1% (5/41) while the rest belonged to pediatric and surgical wards. Although there was no significant increase in the number of pediatric admissions during this period, the mean number of candidemia episodes increased from 1.8 to 5 per year during the period 2001-2006 to 2007-2012, respectively. *Candida parapsilosis* was the most frequent species isolated (43.9%) followed by *Candida albicans* (31.7%) and an increasing number of other non-*albicans* spp. that was noted over time. Non-*albicans* candidemia increased from 43% in the period 2001-2006 to 64% in the period 2007-2012. Overall mortality was 72% in the first period and 46% in the second period (p=0.28). Case-attributable mortality was not different between cases due to *C. albicans* or *C. parapsilosis* (33.3% and 39%, respectively). There was a decreasing frequency and no episode of candidemia during the last 3 years in pediatric oncology patients probably due to implementation of prophylactic protocols.

Conclusions: The rate of candidemia continues to increase in children over these 12 years and almost half of the episodes occur in PICU patient population, although there is a trend towards improved mortality. Non- *albicans* species and particularly *C. parapsilosis* occur with increasing frequency.

Keywords: Candidemia; children; PICU; pediatric oncology

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Laboratory diagnosis of tinea capitis: A Romanian centre experience

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Background. Tinea capitis is a common dermatophyte infection of the scalp and hair shafts (1, 2). Accurate diagnosis is essential for successful treatment (3). In this study, we aimed to determine the frequency of tinea capitis in patients sent for investigation to the Mycology Laboratory of "St. Spiridon" Clinical Emergency County Hospital Iasi, Romania.

Material and methods. We have performed a retrospective analysis of tinea capitis cases presenting to the Mycology Laboratory of "St. Spiridon" Hospital between January 2013 and December 2016. Diagnosis was confirmed based on KOH wet-mount examination, as well as Wood lamp examination and culture, when requested.

Results and discussions. A total of 336 patients, aged between 1 and 90 years, were investigated for tinea capitis. Following the laboratory tests, diagnosis of fungal infection was confirmed in 27% of cases. Most laboratory-proven cases were found in male children aged 1-14 years (58.2%). The most frequent type of lesion was caused by *Microsporum* species (60.4%). Only one favus case was diagnosed. Both *Microsporum* and *Trichophyton* infections were more common in patients from urban areas (63.7%). Although *Trichophyton* species usually affect boys and girls equally (4), our results show that both *Microsporum* and *Trichophyton* species were found predominantly in boys.

Conclusions. Tinea capitis represents a public health concern. The most frequent causative agent of tinea capitis in our region remains *Microsporum spp*, affecting mainly children. The results of the study show the importance of laboratory tests for proper diagnosis of mycotic scalp infections and subsequent adequate therapy. Clinical findings may be confusing and should be supported by laboratory tests.

Keywords: Tinea capitis, Dermatophyte, *Microsporum*, *Trichophyton*

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A retrospective analysis of tinea capitis in Athens - Greece (2012-2017)

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Background: The study presents the epidemiology of fungal species related to the tinea capitis in the area of Athens. Tinea capitis is a common infection of the scalp hair caused by dermatophyte fungi. After the introduction of griseofulvin, the prevalence of tinea capitis was brought under effective control in Europe and North America. At the same time, the prevalence remains significant in endemic countries in other continents.

Material and Methods: The retrospective analysis (2012-2017) based on records of outpatients who visited the "Andreas Syggros" Hospital (Athens, Greece), a tertiary referral hospital of dermatologic diseases covering more than four million people of the Greek capital (almost half of the national population). Samples were taken by scraping or by using a scalp brush such as a disposable toothbrush or swab. Mycological investigation by conventional methods (direct microscopy and culture on Sabouraud dextrose agar and Sabouraud dextrose agar with actidione) was performed in 937 patients (447 women and 490 men) with clinically suspected tinea capitis

Results and discussions: Positive results were found in 516 patients (345 women and 171 men), corresponding to 55% of the total. From these patients, 137 were immigrants from Balkan, Middle East and African countries. The vast majority of the patients (96%) were children, mainly at preschool and school age and only 4% were adults. The most common clinical presentation was ringworm (85%). Direct examination was positive in 314 cases (60 %). Cultures recovered dermatophytes in 367 cases (71 %). The following dermatophyte species were isolated: *Microsporum canis* (76%), *Trichophyton violaceum* (10%), *T. tonsurans* (5%), *T. mentagrophytes* (5%), *T. soudanense* (2.3%), *M. gypseum* (0.7%), *T. rubrum* (0.6%), *M. audouinii* (0.3%) and *M. ferrugineum* (0.2%). The majority of anthropophilic infections (48 %) were recorded in the examined group of immigrants.

Conclusions: The findings confirmed the presumption that *M. canis* is the leader among the causative agents in tinea capitis in children, but its presence in the etiology of disease in adult patients was very low and nonsignificant. Moreover, anthropophilic dermatophytes was the main etiologic agent in immigrants. Mycological investigation is important in order to select the most appropriate treatment.

Key words: Dermatophytes, Epidemiology, Greece, Tinea capitis

Pichia membranifaciens - a new yeast strain with enhanced antifungal activity for biocontrol technologies

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Background. Many modern approaches for fungal spoilage prevention and for achieving a good agricultural practice are based on using antagonistic microorganism (yeasts, lactic acid producing bacteria) [1; 2; 3].

The aim of the present study is the taxonomical identification of a new yeast strain and the characterization of its antimicrobial activity against phytopatogenic fungi, with future potential applications in biocontrol.

Materials and Methods. The yeast strain, preserved in MICROGEN Culture Collection (CMGB), Faculty of Biology, was identified by conventional taxonomy tests and PCR-RFLP analysis of the ITS1-5,8S-ITS2 region using three endonucleases: *Cfo* I, *Hae* III, *Hinf* I.

Tests were performed to establish the potential of the identified yeast as a biocontrol agent against filamentous fungi (molds) belonging to: *Aspergillus, Alternaria, Rhizoctonia, Botrytis* and *Monilinia*. The antifungal activity screening studies were done by co-cultivation of the yeast strain with the molds on PDA medium (8 days, 28°C). The yeast-molds interactions were also evaluated by inoculation the yeast on radial streaks related to a target filamentous fungus colony, using a comparative analysis with other two yeasts: *Pichia anomala* CMGB112 and *Candida guilliermondii* CMGB44 [4].

Results and Discussions. According to the conventional tests, the analysed yeast strain belongs to *Pichia membranifaciens* being able to grow at 20, 28 and 37°C and in presence of 50% glucose. Asci with 2-4 ascospores were observed. The taxonomic classification was confirmed by PCR-RFLP, the strain being named *P. membranifaciens* CMGB76.

The screening tests showed that *P. membranifaciens* CMGB76 had the highest antifungal activity against *A. ochraceus* (75,6%), good activity against *A. flavus* GE2 (70.5%) and reduced against *A. flavus* TE11 (48.8%). The interaction studies revealed yeast inhibition of conidia proliferation of *B. cinerea* > *R. solani* > *A. mali* > *A. carbonarius*. An exception was observed in the case of *Monilinia* sp. who invaded *C. guilliermondii* CMGB44.

Conclusions. The newly identified yeast strain *P. membranifaciens* CMGB76 proved important potential as a biocontrol agent against phytopathogenic fungal infection. Further studies will be performed concerning its effect on mycotoxin production and the antimicrobial activity in presence of a wider range of microorganisms.

Keywords: *Pichia membranifaciens,* phytopathogens, antifungal, biocontrol

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Comparison of two different commercial antifungal susceptibility methods with CLSI for unusual and emerging non-*albicans Candida* species

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Background. In recent years, an increasing number of uncommon *Candida* species less susceptible to azoles and echinocandins have been reported to play an important role in invasive fungal infections. In this study we aimed to investigate the antifungal susceptibility profiles [wild type (WT), non-wild type NWT)] of such strains using more easy, affordable and commercial alternative tests and to detect their agreement with CLSI methodology.

Materials and Methods. Antifungal susceptibility of 60 uncommon non-*albicans Candida* clinical isolates to fluconazole (FCZ), voriconazole (VOR), amphotericin B (AMB), caspofungin (CAS) was tested using gradient test (AB Biodisk) and Sensititre Yeast One (SYO) (TREK Diagnostic Systems) then compared to the gold standard, CLSI broth microdilution (BMD). The essential agreement (EA) was defined as discrepancies between MIC values no more than ±2 twofold dilutions. *C.lusitaniae* (n: 25), *C.intermedia* (n: 12) were grouped as Group 1 and 2, respectively. Group 3 was constituted by 23 isolates of *C.famata* (n:4)*, C.dubliniensis* (n:4)*, C.sake* (n:4), *C.inconspicua* (n:2)*, C.lipolytica* (n:2)*, C.pulcherrima* (n:2)*, C.utilis* (n:2), *C.catenulata* (n:1)*, C.mellibiosica* (n:1) and *C.pelliculosa*

(n:1). Additionally we detected ECOFF values (WT, NWT) of 30 isolates belonging to *C.lusitaniae*, *C.dubliniensis* and *C.pelliculosa*.

Results and Discussion. According to CLSI, all of 25 *C.lusitaniae* isolates are found to be WT for FCZ, VOR, AMB and only 8 were WT for CAS. Out of 4 *C.dubliniensis* 2 were WT for FCZ, 3 WT for VOR, 4 WT for AMB but, all of them were NWT for CAS. One *C.pelliculosa* strain was WT for both FCZ, VOR while NWT for CAS; AMB ECOFF value is not available in CLSI. Corresponding percentiles of the two commercial tests with CLSI BMD are given in Table 1.

Conclusion. According to our results, we can suggest that SYO can be used for detecting AMB susceptibility of uncommon *Candida* species as well as susceptibility to azoles for *C.lusitaniae*. For caspofungin, neither SYO nor gradient test is recommended due to low levels of agreement with CLSI. Our results will contribute to epidemiological studies for these emerging pathogens potentially resistant to various antifungals.

Keywords: Antifungal susceptibility, non-*albicans Candida* species

Table 1. *Corresponding percentiles of two antifungal susceptibility methods with CLSI BMD*

Method	Species	Antifungal	Correspondence %
Sensititre Yeast One	***C.lusitaniae***	Fluconazole	80
	n: 25	Voriconazole	96
		Amphotericin B	100
		Caspofungin	12
	C.intermedia	Fluconazole	67
	n: 12	Voriconazole	75
		Amphotericin B	83
		Caspofungin	25
	Others	Fluconazole	61
	n: 23	Voriconazole	48
		Amphotericin B	96
		Caspofungin	26
Gradient Test	***C.lusitaniae***	Fluconazole	56
	n: 25	Voriconazole	92
		Amphotericin B	64
		Caspofungin	24
	C.intermedia	Fluconazole	58
	n: 12	Voriconazole	83
		Amphotericin B	58
		Caspofungin	33
	Others	Fluconazole	70
	n: 23	Voriconazole	65
		Amphotericin B	61
		Caspofungin	39

Comparative evaluation of E-test and Sensititre YeastOne with CLSI microdilution method for antifungal susceptibility testing of bloodstream yeast isolates.

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Background: The aim of this study was to compare E-test and Sensititre YeastOne with CLSI standard method in order to evaluate these commercially available tests for routine testing of antifungal agents against the most frequently isolated Candida species isolated from blood cultures.

Materials and Methods:

Candida species isolated from the blood cultures of patients were identified using conventional methods and DNA sequencing analysis.

In vitro antifungal susceptibility of isolates for amphotericin B, flucytosine, fluconazole, ketoconazole, itraconazole, voriconazole, anidulafungin, caspofungin, posaconazole, micofungin were determined by Clinical and Laboratory Standards Institute (CLSI, M27-A3) reference broth microdilution method (BMD), E-test strips (Biomeriux, France), and Sensititre YeastOne panels (TREK Diagnostic Systems) that were performed according with the manufacturer's recommendations (1-4).

Results and discussions

The species distribution of the isolates was as follows: C. albicans (n=15), C. parapsilosis (n=15), C. glabrata (n=14) and C. krusei (n=5).

For all Candida strains, ranges of EA and CA according to antifungal in the Yeastone and E-Test, Yeastone and MD methods, and Etest and MD method were found in 72-100% and 92-100 %; 50-100% and 42-100%; 60-100% and 66-100% at 24 hours, respectively. The highest EA and CA for all Candida strains were also determined in both amphotericin B (100%) among Yeastone and MD method for both 24 and 48 hours. In addition, the lowest EA and CA were found in Yeastone and MD methods with itraconazole at 24 hours and the Etest and MD method at 48 hours with ketoconazole.

The highest very major errors (VME), major errors (ME), and minor (MIN) errors for all Candida strains were found in among Etest and MD methods, Yeastone and MD methods, and Yeastone and E-Test methods, respectively.

Conclusion: It was determined that E-test and YeastOne methods compared with the CLSI reference method for determining the susceptibility of *Candida* spp. are easier, feasible, and reproducible method of susceptibility testing. However, further evaluation of their performance for determining the MICs of azoles, particularly for ketoconazole, itraconazole, voriconazole is needed.

Key Words: antifungal susceptibility testing, *Candida* spp., The E-test method, Sensititre YeastOne colorimetric antifungal panel

***Table**. Essential agreement (EA), categorical agreement (CA), very major errors (VME), major errors (ME), and minor (MIN) errors to antifungals of 50 isolates of Candida spp. as determined by the CLSI reference standard ,the E-test and the YeastOne antifungal panel, using clinical breakpoints and epidemiological cutoff values.*

Species (no.)	Antifungal agent	Yeastone/E-test					Yeastone/BMD					E-test /BMD				
		EA[a] (%) 24/48	CA[b](%) 24/48	% of errors[c] Yeast/Etest			EA(%) 24/48	CA(%) 24/48	% of errors BMD/yeast0ne			EA(%) 24/48	CA(%) 24/48	% of errors BMD/Etest		
				MIN	ME	VME			MIN	ME	VME			MIN	ME	VME
Candida (50)																
	Amphotericin B	72/92	100/100	0/0	0/0	0/0	100/100	100/100	0/0	0/0	0/0	54/78	100/100	0/0	0/0	0/0
	Flucytosine	-	-	0/0	0/0	0/2	94/82	100/96	0/0	0/0	0/2	-	-	-	-	-
	Fluconazole	100/94	96/68	1/10	0/3	0/3	84/90	96/98	8/4	0/4	0/1	90/88	84/82	6/4	0/0	0/7
	Itraconazole	88/78	96/88	0/0	0/3	2/6	50/62	42/64	5/6	0/0	16/14	68/78	66/64	5/6	0/0	12/12
	Ketoconazole	-	-	-	-	-	-	-	-	-	-	52/52	68/50	0/4	0/0	16/19
	Posaconazole	96/92	96/86	0/0	0/0	0/7	-	-	-	-	-	-	-	-	-	-
	Voriconazole	96/96	92/92	0/1	1/2	3/1	72/82	62/72	7/9	0/1	12/4	70/76	70/64	7/9	0/1	8/8
	Anidulafungin	76/90	100/100	0/0	0/0	0/0	-	-	-	-	-	-	-	-	-	-
	Caspofungin	92/92	96/82	2/6	0/3	0/0	-	-	-	-	-	-	-	-	-	-
	Micafungin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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Antifungal activity of chitosan against clinical isolates of Candida spp.

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Background. Chitosan is a linear polysaccharide composed of D-glucosamine (deacetylated unit) and N-acetyl-d-glucosamine (acetylated unit) (1,2). It is obtained by deacetylation of chitin, the primary polysaccharide component of crustacean exoskeletons with alkaline sodium hydroxide (1). The antimicrobial properties of cationic polymers have been known for a long time, but biocompatibility, biodegradability and lack of toxicity have led to the use of chitosan in various fields (3,4). The limitations are represented by water insolubility, high viscosity and protein coagulation at high pH (1,4). The purpose of this study was to investigate the *in vitro* antifungal activity of low molecular weight chitosan (LMWC) on Candida species compared to some classical antifungal drugs: Nystatin, Ketoconazole, and Itraconazole.

Materials and methods. Solubilization of chitosan was achieved using 1% solution of acetic acid at pH 3.5 and continued stirring for 5 hours. Antifungal susceptibility testing was performed using the disk diffusion technique combined with well technique, using different concentrations of chitosan (0,1%, 0,2%, 0,5%). A total number of 16 Candida spp. strains, represented by *C. albicans, C. krusei, C. kefyr, C. parapsilosis* and *C. famata* were included in the study.

One hundred microliters of each yeast suspension (0.5 McFarland) were plated on SDA. The plates were then allowed to dry and a 4-mm-well was cut in the agar. The solution of chitosan was both used to fill the well and placed on a filter paper disk. The volume of chitosan solution used in both cases was 10 µl. The antifungal disks were also put on the agar surface and the plates were incubated at 37°C for 24-48 h.

Results and discussions. The results demonstrated the effectiveness of chitosan compared with the usual antifungal drugs because of an increased efficiency, with the mean of the inhibition area of 12.08 mm for 0.1% concentration, 12.34 mm for 0.2% and 13.56 mm for 0.5% respectively. The technique using chitosan solution placed in the well is less recommended compared to the filter paper disk.

Conclusions. The study concluded that the use of chitosan opens up new therapeutic perspectives to combat candidiasis due to reduced toxicity and good overall efficiency compared to classic antifungal drugs.

Keywords: chitosan, Candida, antifungal susceptibility testing.

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In vitro antifungal susceptibility of *Candida glabrata* clinical isolates against six antifungals

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Background. Although *Candida albicans* remains the most common etiological agent overall, non-*albicans Candida* species are increasingly encountered. *C. glabrata* is an important fungal pathogen that causes life-threatening infections and limits the antifungal therapeutic options due to its resistance or reduced susceptibility to the azole agents and the ability to develop resistance to both azoles and the echinocandins (1). We aimed to evaluate antifungal susceptibility of clinical *C.glabrata* isolates against six antifungal drugs.

Materials and methods. A total of 127 non-duplicate *C. glabrata* isolates from clinical specimens such as blood, urine, lower respiratory tract, and tissue were included. Previously, all isolates had been identified by using a commercial assimilation test (API 20C, BioMerieux). Antifungal susceptibility of *C. glabrata* isolates to caspofungin (CAS), anidulafungin (AND), amphotericin B (AMB), fluconazole (FLU), voriconazole (VOR) and posaconazole (POS) was detected by reference broth microdilution method according to Clinical and Laboratory Standards Institute guidelines (CLSI M27-A3 and -S4) (2, 3).

Results and discussions. Minimal inhibitory concentration (MIC) ranges, MIC50 and MIC90 values were ≤0.015-0.06, 0.015 and 0.03 g/L for CAS; ≤0.015-0.06, ≤0.015 and 0.015 g/L for AND; 0.5-2.0, 1.0 and 2.0 g/L for AMB; 1.0-≥64.0, 4.0 and 8.0 g/L for FLU; 0.06-≥16.0, 0.25 and 0.5 g/L for VOR; 0.06-≥16.0, 0.5 and 1.0 g/L for POS, respectively. According to CLSI interpretive breakpoints (3), all *C. glabrata* isolates were susceptible to CAS and AND, and four (3%) of isolates were resistant to FLU (MIC≥64 g/L). *C. glabrata* specific interpretive MIC breakpoints for AMB, VOR and POS have not been established in CLSI. However, AMB MICs for 24 of isolates were 2 g/L, VOR MICs for four isolates and POS MICs for five isolates were ≥4 g/L. VOR and POS MICs were ≥4 g/L for all of the FLU resistant isolates (n=4).

Conclusions. Although, echinocandin resistance has been reported more often among *C. glabrata* isolates (almost 10% at selected institutions) (4), we didn't detect; all of *C. glabrata* isolates were susceptible to echinocandins in this study. *C. glabrata* is known to exhibit reduced susceptibility or resistance to FLU (almost 10-30%) and the other azoles (1, 5). FLU resistant *C .glabrata* rate was low (3%), but they also had high MICs for VOR and POS in our study.

Keywords: *C.glabrata*, antifungal susceptibility, echinocandin, azole

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Two new yeast strains from traditional Romanian dairy products with anti-*Candida* activity and probiotic abilities

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Introduction: Yeasts are *ubiquitous microorganisms isolated from food, feeds, environment, industry or clinical samples. Nevertheless, there are few yeast genera present in dairy products, such as Kluyveromyces and Issatchenkia, with specific metabolic or antimicrobial abilities [1; 2].*

The present study deals with the identification of two new yeast strains isolated from Romanian traditional dairy products and the characterization of their anti-Candida activity and ability to produce lipases with possible probiotic applications.

Material and Methods*: The two yeast strains isolated from milk and cheese (Ialomita, Romania) were identified using biochemical tests (Biolog System) and the PCR-RFLP analysis of the ITS1-5.8S rRNA-ITS2 region using the endonucleases Cfo I, Hae III, Hinf I, Msp I.*

The antimicrobial tests were performed by screening the killer activity against six *Candida* strains: *C. albicans* ATTC10231, *C. parapsilosis* CBS604 and *C. krusei* CMGB94, respectively, *C. albicans* CMGB-Y13, *C. parapsilosis* CMGB-Y3 and *C. krusei* CMGB-Y8 (from urogenital infections).

The ability to produce lipases was evaluated by observing tributyrin hydrolysis on solid medium.

Results and Discussions: *The phenotypic phylogeny based on biochemical characterization allowed the preliminar identification of the two strains as Kluyveromyces marxianus (K. marxianus 230) and Issatchenkia scutulata var. exiguus (I. scutulata var. exiguus 231). The size of the ITS1-5.8S rRNA-ITS2 amplicons and the restriction profiles were compared with those from the scientific literature [3] and confirmed the taxonomic classification of the two strains.*

The strain I. scutulata var. exiguus 231 had high killer activity against C. krusei CMGB94 and *C. albicans* CMGB-Y13 (dose dependent susceptible to fluconazole), while *K. marxianus 230 inhibited the growth of C. parapsilosis* CBS604 and *C. krusei* CMGB-Y8. In this case, the microscopical observations showed *Candida* cells with large vacuoles due to stress conditions induced by the killer toxin.

Both strains produced lipases that hydrolysed tributyrin liberating the butyric acid, a beneficial compound for human health [4].

Conclusions: The two new strains *K. marxianus 230 and I. scutulata var. exiguus 231 present antimicrobial activity against pathogenic Candida strains and good ability of lipase synthesis for probiotic use proving* high potential for further biomedical applications.

Keywords: *Kluyveromyces*, *Issatchenkia*, dairy, anti-*Candida*, probiotics

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Comparison of four methods for the evaluation of in vitro susceptibility testing of dermatophytic isolates

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Background: Infections caused by dermatophytes affect a high percentage of the population. Antifungal susceptibility testing (AST) offers information about the susceptibility profiles of the pathogens, documentation of the appropriate treatment and reduction of the cost. The slow growth rate of these fungi and their poor sporulation are factors that delay and affect the performance of the AST. The proposed methods by the CLSI or the EUCAST are both laborious for the everyday routine. However, there are alternative applications that propose the use of an inoculum consisting of a conidia-mycelium mixture (1,2) or even from plain mycelia (3), as well as the use of resazurin in order to facilitate the reading (4). The aim of this study was to compare these approaches to the EUCAST method (5) in order to evaluate their performance.

Methods: Three alternative methods of dermatophytic AST were compared to the EUCAST proposed methodology for conidia forming moulds. The methods under evaluation were a) a fragmented mycelia method, b) the EUCAST method with the addition of resazurin sodium salt solution and c) the fragmented mycelia method with the addition of resazurin sodium salt solution. The susceptibility of twenty dermatophytic isolates (8 *Trichophyton interdigitale*, 6 *T. rubrum* and 6 *M. canis*) was tested against griseofulvin, terbinafine, fluconazole and itraconazole.

Results: The essential agreement between the methods was calculated in percentages. Data analysis revealed sufficient overall essential agreement of the methods with the addition of resazurin to the initial "uncoloured" methods (98.5% and 97.2% for the EUCAST or the fragmented mycelia method). The fragmented mycelia method exhibited a relatively sufficient overall essential agreement to the EUCAST method (88.9%) but not a satisfactory correlation. The mean MICs (by the EUCAST method, in μg/mL) for the twenty isolates were 1.78 for griseofulvin, 0.034 for terbinafine, 25.2 for fluconazole and 0.57 for itraconazole.

Conclusions: The addition of resazurin sodium salt solution can facilitate the reading and provide a more objective evaluation. The fragmented mycelia method could serve as an alternative that due to technical reasons should be applied only in cases of poor or no sporulating dermatophytes.

Keywords: dermatophytes, susceptibility, EUCAST, resazurin, fragmented mycelia

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VETERINARY NUTRITIVE SUPPLEMENT FOR REDUCTION THE MYCOTOXIN CONTAMINATION IN SWINE

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Ochratoxin, a mycotoxin produced by *Aspergillus* and *Penicillium* species, raises serious problems for the poultry and swine industries because monogastric animals lack the ability to degrade ochratoxin rapidly. Ochratoxin appears in stored grains and its impact is greatest in temperate climates where most of the world's grain is produced and stored (1). One of the strategies for reducing the exposure to mycotoxins is to decrease their bioavailability by including various adsorbing agents in feed (2). The paper refers to an innovative veterinary supplement (3) based on essential oils (EO) and diatomaceous earth (DE) with applications for the nutrition and protection of the swine against contamination with mycotoxins. The original product consists of 53-58% mineral adsorbent (for example the DE from Adamclisi quarry), 2-3% EOs showing antifungal action (such as the oregano EO), 3-5% soy protein isolate, 0.7-0.9% Ca $OH)_2$, 0.1-0.2% KOH, 37-38% milk whey. Laboratory *in vitro* testing of oregano EO against toxigenic *Aspergillus* and *Penicillium* species demonstrated its fungistatic activity by totally inhibiting the growth of mentioned fungi (4). The preparing process includes the alkaline thermo-hydrolysis of the protein isolate followed by immobilization of the nutritional elements and bioactive EO in hydrogel, encapsulation and granulation of the product (5). Preliminary results obtained by IBNA Balotesti administering the new nutritional supplement on piglets have shown efficacy in stimulating IgG synthesis, the antibody that provides long-lasting immune response, increasing the body's resistance to infections (6). The product and the procedure have the following advantages: (i) Synergistic action of DE with EO, which performs antimycotoxigenic and fungistatic action simultaneously with the fortification of the organism by organo-minerals (calcium chelates, organic potassium) and protein intake; (ii) The resulting granules have superior adherence due to the porosity of DE, the pleasant vegetable odor; (iii) The composition is based exclusively on non-toxic, inexpensive and affordable natural ingredients; (iv) The production process involves simple, clean, energy-efficient and waste-free technology, being in perfect accord with the present global trend to a sustainable development of the bio economy.

***Key words**:* Toxigenic fungi and mycotoxins, DE, EO, swine, veterinary supplement

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Total aflatoxins occurrence in spices from the Romanian market

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Background. Due to their natural origin, spices are likely to accumulate mycotoxins (1). The present research addresses a matter of practical interest in assessing the incidence of total aflatoxins in the most used spices, marketed by 4 companies in Romania (A, B, C, and D).

Materials and methods. The prepacked spices analyzed were: pepper, sweet paprika, cumin, cinnamon and ginger. The level of aflatoxin contamination was highlighted by half-quantitative immunochromatographic tests (RidaQuick aflatoxin) in 18 spices samples (4 spices x 4 companies plus 2 ginger samples from C and D companies). Spices suspensions were also inoculated on Petri plates containing DG18-L1 Agar. Four types of fungal colonies were isolated and passed on Malt Extract Agar. To identify and characterize toxigenic fungi, the Biolog MicroStation System MicroLog was used.

Results and discussions. From the "color development time on the strip-ppb aflatoxin" regression curve, the levels of mycotoxins were calculated. Aflatoxin levels were exceeded for all spices, the smallest being in pepper (B, C, D; 3-7 ppb). The highest aflatoxins amounts were in cumin (4-39 ppb), followed by ginger (4-24 ppb), paprika (4-14 ppb) and cinnamon (8-9 ppb). The development of fungi colonies was not proportional to the aflatoxins levels (2, 3). Four types of molds, identified as *Aspergillus flavus*, *Aspergillus niger*, *Mucor racemosus* and *Lichtheimia corymbifera*, developed colonies on Petri plates inoculated from pepper (C; 4 ppb) and cinnamon (A, B, C; 8-9 ppb). In the cumin sample (B; 39 ppb), only yeasts colonies have grown. It was confirmed that there are mycotoxins in spices, even the fungi can not be isolated anymore (4). Overall, spices marketed by A company showed the most abundant fungal contamination, and those marketed by D company, the lowest.

Conclusions. Spices can be significant vectors for aflatoxin transfer in food. Mycotoxicological risks are particularly amplified when extracting active ingredients from spices (5), where large quantities of those are used. An expensive chromatographic method, such as HPLC mycotoxins detection, is unnecessary because the immunochromatographic assay demonstrated its efficiency (lower detection limits-4 ppb, than the permissible levels of aflatoxins in food-5 ppb).

Key words: total aflatoxins, spices, toxigenic fungi, immunochromatography

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Aflatoxin contamination of various foods of vegetal origin

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Background. Aflatoxins are a group of secondary metabolites produced after the growth phase of *Aspergillus* toxigenic molds: *A. flavus, A. parasiticus, A. niger, A. versicolor, A. wentii*. There are also several *Penicillium* species able to produce aflatoxins (*puberulum, variables, citrinum*), as well as *Rhisopus* species (2).

Food contamination with aflatoxins occurs when toxigenic species successfully colonize a product, develops and finds appropriate conditions for the production of toxins. Chemically, aflatoxins are 18 polycyclic bisulfite compounds, which emit strong fluorescence in ultraviolet light (365nm). The most important aflatoxins are: B1, B2, G1, G2, M1, M2, B2a, parasiticol, aflatoxicol, aflatoxicol H1, aflatoxicol P1, aflatoxin Q1. Aflatoxins B1 and B2 generate blue fluorescence, while G1 and G2 generate green fluorescence. Other four aflatoxins: M1, M2, B2a, and G2 α, are produced in small amounts. Aflatoxins are very stable compounds in food substrates and resists extreme pH values, ≥ 3 and > 10, in UV radiation and in the presence of oxidizing agents. They are extremely thermostable.

The results of mycotoxicological investigations in different countries show that the incidence of aflatoxins in vegetal substrates is variable, depending on the substrate, its humidity, aeration degree and climatic conditions, so that mycotoxins can only be controlled by a sustained micotoxicological surveillance program, especially at the level of storage and processing of raw materials (1).

Materials and methods. The sampling of the food samples and the methods of analysis were in accordance with the provisions of Regulation (EC) No. 401/2006 of the EU Commission, using the MaxSignalTM Total Aflatoxin ELISA - Bio Scientific test (3). For interpretation, the values obtained were reported to the provisions of Regulation (EC) No. 1881/2006 laying down the levels of aflatoxins admitted in food (4).

Results and discussions. As a result of the analyzes made on 22 assortments of food of vegetal origin, all values were found to be within the admissibility parameters, however, higher values of total aflatoxins occurred in walnut kernel - 1.67 ± 0.1540 μg/kg, - 1.43 ± 0.059 μg/kg, dehydrated apricots and half peanuts - 0.67 ± 0.1320 μg/kg and nutmeg aflatoxin B1 - 0.99 ± 0.1844 μg/kg and brown raisins – 0.9 ± 0,1676 μg/kg. The lowest total aflatoxins content were detected for the following products: dried apricots - 0,23 ± 0,02 μg/kg, dried fruit mix - 0,23 ± 0,021 μg/kg, prunes - 0,10 ± 0,04 μg/kg. In the case of aflatoxin B1, the values also fall into the admissibility values, with higher values for the following foods: nutmeg - 0,99 ± 0,1844 μg/kg, brown raisins - 0,9 ± 0,1676 μg/kg. For aflatoxin B1, most of the results were undetectable: dehydrated apricots, dry fruits, dry plums, dehydrated figs, etc.

Conclusions. The obtained values demonstrate the necessity of testing foods of plant origin for the detection of total aflatoxins and B1, taking into account that high or above the limit of admissibility, exert undesirable biological actions on human health following consumption of contaminated food: hemorrhagic syndrome, hepatosis, nephrosis, decreased fertility, increased susceptibility to infection as a result of damage to immunogenic mechanisms, etc.

Keywords: *foods of plant origin, pathogenic fungs, total aflatoxins and B1, expertise.*

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Antifungal activity of sage (*Salvia Officinalis L.*) essential oil against *Aspergillus flavus* growth and aflatoxins production in corn

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Background. According to International Organization for Standardization, an essential oil (EO) represents a mixture of terpenes and terpenoids compounds obtained from aromatic plants by hydro-distillation [1¸2]. Their lipophilic property and low molecular weights are the main causes for the antifungal activity of essential oil components due to morphological alterations on the fungal hyphae [3]. It is well known that sage EO is used in food industry as a spice, or as an additive for flavoring. Also there is a lot of scientific data that show a relation between the concentration of some sage biologically active substances such as terpenes, terpenoids and their antifungal action [4]. The aim of this study was the in vivo investigation of the Salvia officinalis L. volatile oil and its principal compounds α and β- thujone, 1¸8-cineole against Aspergillus flavus growth. Furthermore, the influence of sage EO on the total aflatoxins production in corn was studied.

Material and methods. The experiments were carried out using a series of sixteen glass jars each one containing 100 g of corn beans (contaminated with $1x10^{12}$ spores/g A. flavus). The first fifteen glass jars were treated with pure α and β- thujone, 1¸8-cineole and 5, 50 and 100 ppm of gaseous sage EO. The incubation period was establish at 25ºC for 7, 14, 21, and 30 days respectively. Fungal growth inhibition was monitored by spectrophotometric determination of the optical density at 620 nm. The corn aflatoxins content was determined by ELISA assay.

Results and discussions. *Aspergillus flavus* showed a higher sensitivity to sage EO then to pure substances. At 50 ppm sage EO, the hyphal reduction growth was 0.5 log, whereas using α and β- thujone, 1¸8-cineole the reduction growth were 0.2 and 0.4 respectively. Comparing with the control, the fungal reduction growth was 0.1¸ 0.5 and 0.6 logs when contaminated corn was treated with 5, 50 and 100 ppm of gaseous EO. Considering the total aflatoxin production at 30 days incubation with sage EO's, were detected 2.54, 0.76 and 0.56 ppb total aflatoxins comparing with 25.4 ppb in the control samples.

Conclusions. The experimental results render *Salvia officinalis* EO a promising candidate to counteract the growth of and possible aflatoxin production by *A. flavus* in corn.

Keywords: sage, essential oil, terpenoids, aflatoxins, corn

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A comparative study concerning results interpretation of mycological exam of feeds according to Romanian and German legislation

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Background. The management of feed quality concerning the moulds and yeasts burden is very important for evaluation of animal welfare and health and the quality of products from animal origin.

Materials and methods. The study consisted in mycological examination of 343 samples represented by feeds from comercial and non-professional animal holdings from Western Romania and its aim was to evaluate the feed quality. The quantitative mycological exam was performed according to ISO standard 21527-1/2008 and for examination of morphological structures, wet mounts with Lactophenol Cotton Blue were used. The interpretation of the results was carried out according to: *Order of MAAP and MSF no. 249/2003, Annex no. 6 point II - The microbiological limit-value of feed* (Romania) and *The indicative values for saprophytic and alterative microorganisms as indicators for evaluation of feed quality made by Association of Institutes for Research in Agriculture (VDLUFA)/2011* (Germany).

Results and Discussions. According to Romanian legislation, the results of mycological exams can not be interpreted for 310 samples because of lack of limits concerning the charge of yeasts and moulds for fodder ans silos. According to VDLUFA/2011 report, the interpretation can be performed for all samples (Table 1).

The maximum limits for quality I mentioned in the VDLUFA/2011 report are more permissive than the maximum limits allowed by MAAP and MSF Order no. 249/2003, thus the comparative view was as follows: cereals 86.44% versus 54.24%; mixed fodder 98.73% versus 81.86%; soy and sun-flower meal 75.0% versus 12.25%.

Conclusions. The report issued by VDLUFA/2011 covers the sanitation assessment of all types of feed for animal species of economic interest, by age and quality classes. Taking into account the possibility of intra-Community trade, it is desirable that the interpretation of results of mycological exams to establish feed quality should be done in a unitary way, requiring the VDLUFA/2011 report to be adopted as national legislation or European standard.

Keywords: yeasts, moulds, interpretation, animal welfare

Table 1

Comparative interpretation of the results		Matrix and number of samples				
		Raw materials (cereals) n=59	Mixed fodder n=235	Soy and sunflower meal n=16	Fodder (hay) n=21	Silos n=12
% according to Romanian legislation (maximum limit admitted for toxigenic fungi)	Over the maximum admitted limit	45.76 %	19.14%	87.75%	-	-
	Under the maximum limit admitted	54.24 %	81.86 %	12.25%	-	-
% for each category of quality according to German legislation	Category of quality I	86.44 %	98.73%	75.0%	90.48 %	75.0 %
	Category of quality II	11.86 %	1.27%	25.0%	9.52 %	25.0 %
	Category of quality III	1.70 %	-	-	-	-
	Category of quality IV	-	-		-	-

Solid Phase Extraction coupled with Ultra High Performance Liquid Cromatography and Fluorescence Detection for aflatoxins analysis in wines

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Background: Wine may be an important source of human exposure to mycotoxins [1], of which aflatoxins represent biological risk factors for the public health by considering their nephrotoxic, hepatotoxic, teratogenic and carcinogenic action. The best methods for reducing mycotoxins contamination of the wines consist in efficient preventive control measures during grapes harvesting as well as over the main stages of winemaking. Therefor, significants efforts are needed for expanding more efficient analytical methods.

Materials and Methods: This study purpose was to develop an accurate method for the mycotoxins detection [2] and to analyze the aflatoxins (B_1, B_2, G_1, G_2) in wines, by using pre-concentration of the sample through solid phase extraction (SPE) coupled with separation by ultra-high-pressure liquid chromatography (UHPLC) and detection with fluorescence detector (FLD). Wine samples were obtained through traditional fermentation method for wine-making techniques, in 2016, at the local didactical farm of the University of Agricultural Sciences and Veterinary Medicine "Ion Ionescu de la Brad" Iasi, in conditions of controlled cultural and enological practices.

Results and Discussions: The validation and analytical performance of the proposed method was tested in terms of linearity (regression coefficient: 0.9023-0.9855), limits of detection (LOD) between 0.59-1.61ppb and accuracy (recovery) between 83.18–113.24%. Different types of wine samples were analysed: dry white wines (Zghihară, Aligote, Fetească Albă, Fetească Regală), demi-sweet white wines (Traminer, Chardonay) and red dry wines (Fetească Neagră, Merlot, Cabernet Sauvignon). An increased susceptibility to aflatoxins contamination has been highlighted in all red-dry wines (10-25ppb aflatoxin B_1), in all white demi-sweet wines (10-20 aflatoxin B_1) and in a dry-white wine sample (11ppb aflatoxin B_1) when compared with European Commission Regulation that is setting the maximum levels of certain contaminants in foodstaffs [3].

Conclusions: The current study proposes a specifically SPE-UHPLC-FLD method as being both rapid and sufficiently quantitative for the assessment of aflatoxins in wines. This study pointed out a varietal susceptibility to mycotoxins contamination, since all types of wines samples were produced from grapes harvested in the same aria of NE region of Romania by using the same winemaking technology.

Keywords: SPE-UPHC-FLD method, mycotoxins, aflatoxins, wine

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Evaluation of three isolates of *Microsporum canis* from cats with dermatophytosis by *in vitro* hair perforation test

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Background. Dermatophytes are recognized as keratinophilic fungi exhibiting the enzymatic ability to attack and utilize keratin from skin, hair, nails, hoofs and horns [1]. Earlier studies showed that *in vitro* hair perforation could be used as a supplemental test for identification and differentiation of these superficial pathogens in human and animals [2,3].

Materials and methods. Three isolates of *Microsporum canis* from three young domestic cats with dermatophytosis were evaluated. Ten days old cultures obtained from each sample were used for test procedure. Keratin substrate consisted in sterilized child hair segments placed in three mini-Petri dishes with *Czapek-Dox* agar (5-10 hair segments/plate). These plates were inoculated with fragments of colonies of *Microsporum canis* isolates and subsequently incubated at 27°C for 21 days. Hair segments overgrown with mycelium were removed at 7 days interval, mounted in a drop of lactophenol cotton blue or lactophenol, and examined microscopically for the presence of hair perforation showed by morphological changes in hair structure caused by tested fungi [4].

Results and discussions. All three tested isolates of *Microsporum canis* revealed the ability to degrade human hair, with a progressive activity of hair perforation correlating with time of incubation (maximum level reached at 21 days of incubation). Perforation was associated with several micro-morphological changes of hair as: cuticle lifting, cortical erosions, production of different perforating organs in shape and size (pin-head shaped, finger-glove shaped, icicle-shaped, tunnels fissures, narrow and broad, short and long), penetrating into the hair cortex and medulla [4,5].

Conclusions. Our results demonstrated that the isolates of *Microsporum canis* from cats have got the ability to degrade human hair, with an extensive hair disruption observed at 21 days of incubation. Moreover, the keratinolytic activity was accompanied by an increased conidiogenesis with numerous macroconidia.

Keywords: *Microsporum canis*, cat, human hair, perforation

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Evaluation of chemical composition and antimicrobial activity of three essential oils

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Background. Starting from the fact that the pharmacological and therapeutic properties of an essential oil are given by its chemical components which differ according to the plant chemotypes, we intended to determine the content in volatile compounds of three essential commercial oils and test their antimicrobial effect.

Materials and methods. Three commercially available essential oils - namely laurus (*Laurus nobilis*), cloves (*Syzygium aromaticum*), and thyme (*Thymus vulgaris*), were used in the study. To characterize the chemical composition of the essential oils, an instrumental analysis of the samples was performed using Agilent Technology 7820A gas chromatograph (AGILENT Scientific, Santa Clara, CA, USA) coupled with MSD 5975 mass spectrometer and equipped with a DB WAX capillary column (30 mx 250 μm x 0.25 μm). The NIST Spectrum Library was used to identify volatile compounds. Identification was made by comparing the mass spectra with those stored in the NIST 02, Wiley 275 libraries. Antimicrobial effect testing of essential oils was performed by Kirby-Bauer method on Mueller-Hinton Agar. Five type strains were used: Gram negative bacteria (*Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922), Gram positive bacteria (*Staphylococcus aureus* ATCC 29213 and *Bacillus cereus* ATCC 14893), and yeast *Candida albicans* ATCC 10231.

Results and discussions. Thyme essential oil contains as volatile compounds - p-cimen, timol, g-terpinene and α-pinen; laurus essential oil - eucalyptol, β-pinene, α-pinene, α-bergamotene and α-fenchene; clove essential oil - eugenol, cariophenylene and anethole. Concerning their antimicrobial activity, it was shown that the efficacy is higher against Gram-positive bacterial species and yeasts (47±4 mm) compared to the Gram-negative ones (28±3 mm).

Conclusions. Further studies are necessary in order to corelate the chemical composition of various essential oils extracted from various plant chemotypes with their antimicrobial efficacy.

Keywords: thyme, clove, laurus, essential oil, antimicrobial activity

Fungal pneumonia due to Rhizopus microsporus in a captive immunosuppressed Alpaca (Vicugna pacos) from Zoo Park – a case report

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Background. Zygomicoses are fungal diseases that can affect the major animals and human. The most important aspect of this disease is blood vessels involvement, especially the arteries, with angioinvasion by hyphae, causing subsequent thrombosis and necrosis (1).

The predisposing factors for infection include neutropenia, diabetes mellitus or grain overload in animals, iron overload, trauma, corticosteroids therapy and malnourishment.

Materials and methods. A death body of eight years old and 55 kg weight female Alpaca was presented to our Pathology Department for autopsy. The animal died in Zoo Park Bârlad, Vaslui County, after 10 days of corticosteroid (prednisone) and antibiotics (enrofloxacin) therapy for pneumonia. Gross examination and routine histopathology were performed on a large set of tissues.

Twelve hours after the death of the animal, during the post-mortem examination, all specimens were fixed in 10% buffered formalin and embedded in paraffin. Sections of 5µm thickness were obtained, deparaffinized and stained by the Masson trichrome and PAS stains. The qualitative histology was performed from stained sections.

Also, from relevant tissue lesions 5 samples were collected and culture on different media has been performed in order to isolate the pathogens.

Results and discussions. On gross examination, the Alpaca was in poor body condition, with emaciated musculature, indicating that a debilitating disease affected the animal.

The most affected organs seen at the necropsy were the lung and pleural cavities. The lung was increased in volume, with necrotic foci on the dorsal surface and many abscesses in the parenchyma. On the surface of visceral pleura, it was observed small detachable fibrin membranes. The liver was slightly enlarged, pale and friable.

Histologically, in the lungs, a severe necrotic inflammation, with large area of necrosis, rounded by inflammatory cells (macrophages, neutrophils), congestion and hemorrhages, and large territories of diffuse purulent inflammation were observed. Many extracellular, perivascular and intravascular, highly pleomorphic and non-septated hyphae spread in the lung parenchyma and intense acute inflammatory reaction with neutrophils and lymphocytes were observed. The highlights of lesions observed were the invasion of blood vessels, especially pulmonary arteries with secondary vascular thrombosis. No giant cells were observed. The culture on Sabouraud chloramphenicol Agar revealed Rhizopus microspores in 3 of 5 samples.

Conclusions. In mammals, mycoses usually are the manifestation of underlying immune suppression (2,3). Corticosteroids and antibiotic therapy for the previous pyogenic bacterial infection can be considered the real cause of immunosuppression.

Our suppositions about pulmonary mucormycosis raised in the context of immunologic deficiency status of the animal induced by 10-day prednisolone and enrofloxacin therapy, and malnutrition.

Diagnosis was confirmed by culture and morphological identification of the fungal isolate.

Keywords: alpaca, Rhizopus microsporus, corticosteroids, immunosuppression

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Clinical features and diagnosis of *Aspergillus* infections in *Psitacidae*

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Background. Diagnosis of systemic aspergillosis in cage birds implies a correlation between the poor immune status and the permanent exposures to predisposing environmental factors (inadequate biotope, high relative humidity, low quality food) [1]. The aim of this study was to present a series of aspergillosis cases in cage birds and to emphasize the their clinical features.

Materials and methods. The study was conducted on a number of 7 birds, 1 to 10 years old, from *Psitacidae* family referred to our outpatient clinic for diagnosis and treatment: *Psittacus erithacus* (African grey) - 3 cases, *Nymphicus hollandicus* (nymph) - 2 cases, *Psittacula krameri* (Little Alexander) – one case, *Eolophus roseicapiullus* (rosa cockatoo) – one case. The birds exhibited various pathology of digestive tract (ingluvial indigestion, dysphagia, food content in faeces), respiratory tract (sneezing, wheezing breath, runny nose) or skin. All investigated patients lived in individual cages in owners' house as birds of company and they were allowed daily to fly inside. The evaluation of the patients followed a predetermined protocol – parasitological tests (including faecal smears for identification of *Macrorhabdus ornitogaster*), bacteriological and mycological analyses, and antimicrobial susceptibility testing if it's necessary.

Results and discussions. Seven cases of aspergillosis were documented as follows: 5 cases of *Aspergillus fumigatus* infection (including 3 co-infections with *A. niger*) and 2 cases of *Aspergillus clavatus* infection (including one co-infection with *A. niger*). Respiratory signs were predominant and were associated with changes in the structure of the feathers. The bacteriological investigations have proved co-infections with *Proteus* spp. (3 cases), *Streptococcus* spp. (2 cases), and *Haemophillus* spp. (2 cases). The therapeutic scheme was based on a new diet, administration of antibiotics (fosfomycin in association with tylosin or doxycycline) and antifungals (itraconazole or voriconazole) [2].

Conclusions. *Aspergillus* species are opportunistic pathogens for cage birds especially when predisposing factors are encountered. An appropriate diagnostic protocol including laboratory tests is mandatory for a correct management of the disease.

Keywords: *Psitacidae*, aspergilosis, diagnosis.

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Professional and economic justification of analyzing various skin diseases for superficial mycoses - One-year study

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Background: Superficial mycoses are diseases of the skin and adnexa caused mostly by dermatophyte, that's why these mycoses are commonly called dermatophytoses. (1) Superficial mycosis appears with typical clinical presentation, but in atopic or immunocompromised patients and in skin changes treated by corticosteroids local finding could be atypical which could lead to wrong diagnoses and treatments (2,3). The aim of this work is to evaluate professional and economic justification of analyzing various skin diseases for superficial mycoses.

Material and methods: The results of mycological investigation of skin, nail and hair samples of patients in Una-Sana Canton of Federation of Bosnia and Herzegovina during the period May 2016 - May 2017 were analyzed with special attention to referral diagnoses. During that period, 605 samples with duly prepared diagnoses in referrals were processed. The processing of all the samples included making wet mounts with KOH and culturing on Sabouraud dextrose agar (SDA) with antibiotics for 3 weeks. Further identification from culture was made based on morphological characteristics of isolate using lactophenol cotton blue slide mounts.

Results and Discussion: Out of 605 samples, there were 291 (48%) with referral diagnoses indicating Tinea and other diagnoses related to superficial mycoses. In these 291 samples, wet mounts showed positive for 26% and culture for 30.5% cases, with *Candida* representing 7.6%. 312 patients had some other, non-dermatophyte skin disease and were processed as the part of dermatological examination. Only for 12% of these patients wet mounts showed positive for mycoses, for 6.4% showed positive culture for dermatophytes and 9.6% were positive for *Candida spp*. There are 20 *Candida*, out of 30 identified, found in patients with diagnoses dermatitis eczematisata, dermatitis and eczema (66%).

Conclusion: With regard to very small number of positive samples among patients with non-mycotic skin disease, there is no indication to refer these patients routinely for mycotic examination. Referring samples for yeasts, especially for patients with wet dermatoses, should be considered.

Key words: Superficial mycoses, dermatophytoses

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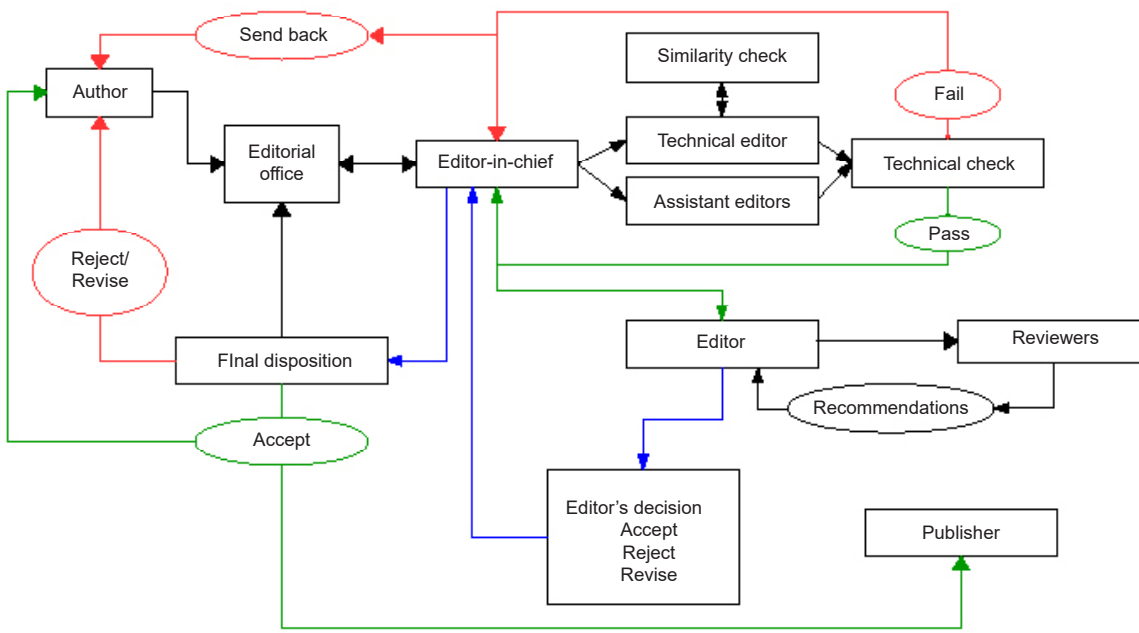

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